

**EFFECT OF MULTIPLE APPLICATIONS OF
CHLORHEXIDINE ON SKIN COLONISATION IN
PRETERM NEONATES – A DOUBLE BLINDED RCT**

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D.M. (NEONATOLOGY)

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CERTIFICATE

This is to certify that the dissertation entitled “**EFFECT OF MULTIPLE APPLICATIONS OF CHLORHEXIDINE ON SKIN COLONISATION IN PRETERM NEONATES – A DOUBLE BLINDED RCT**” is a bonafide work done by **Dr.M.ANITHA** under my guidance and supervision during the period between Nov 2013 – Feb 2014 towards the partial fulfilment of requirement for the award of **D.M.(Neonatology)** degree examination to be held in August 2014 by The Tamilnadu Dr.M.G.R. Medical University, Chennai.

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I solemnly declare that this study title “**EFFECT OF MULTIPLE APPLICATIONS OF CHLORHEXIDINE ON SKIN COLONISATION IN PRETERM NEONATES – A DOUBLE BLINDED RCT**” was my original work in the Department of Neonatology, Institute of child health and hospital for children, Egmore, Chennai under the guidance and supervision of **Prof.J.KUMUTHA,MD.,DCH.**, Professor & Head of the department, Department of Neonatology, Madras Medical College, Chennai. This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai in partial fulfilment of the university requirements for the award of the degree of D.M.Neonatology.

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INTRODUCTION

INTRODUCTION

Preterm birth is truly a global problem. Countries with highest numbers include Brazil, India, Nigeria and the United States of America.¹ In the poorest countries on an average, 12% of babies are born too soon compared with 9% in higher income countries.² Preterm infants due to their immaturity of various organ systems are more likely to be admitted to the neonatal intensive care units (NICU) than their term counterparts who are likely to be by their mother side. During the process of birth, transport to NICU and various procedures of treatment and care, infant's skin gets colonized by flora derived from the body of the mother, other human contacts and various inanimate objects.³

The mechanisms leading to colonization of the skin involve a complex interplay among rapid growth of commensal organisms, the development of the acid mantle, local micro environmental factors such as occlusion and humidity and the choice of exogenous soaps and skin care practices.⁴ Initial colonization depends on the initial organism that colonize at a particular site as well as factors such as type of delivery, the amount of vernix present at birth, the type of nourishment received and the degree of exposure in the hospital environment.⁵ Though microbial colonization begins immediately after birth, it is low initially and the rate increases after 12 hours.³

Most common organisms to colonize among the flora are staphylococcus, streptococcus, acinetobacter, klebsiella and candidal species³. Establishment of healthy skin microbiome may have a role in denying access to infectious microbiobes and help to modulate inflammatory responses. Coagulase negative staphylococcus a commensal bacteria plays a protective role by upregulating the expression of antimicrobial peptides such as human beta defensin-2 in a mature infant. ⁶

In Preterm infants, at 28 weeks the stratum corneum consists of 2 or 3 cell layers. By 32-34 wks there are more than 15 layers of corneocytes equivalent to that of adult skin. Before 32-34 weeks, the thin stratum corneum does not effectively prevent against transepidermal water loss, percutaneous absorption of exogenously applied compounds or invasion of microorganisms. So even the normal flora may cause systemic infections in preterm infants because of the trans - cutaneous access through the immature skin barrier. ⁶

During the first 2 weeks of life the epidermal barrier is immature and functionally compromised. Neonatal infections through the skin occur during this period.^{7, 8} Topical emollients like sunflower seed oil when applied topically augment skin barrier function and reduces the systemic infections in preterm neonates.^{9,10} Topical application of antiseptics until the skin matures could prevent skin colonization and thereby reduce the systemic infections in neonates.

Chlorhexidine is a broad-spectrum antiseptic. It is used frequently for umbilical cord care in neonates.¹¹ It is now being evaluated for topical application to the skin.

In the studies undertaken in the community, a single skin cleansing with 0.25% chlorhexidine resulted in reduction in mortality among low birth weight infants¹². Hospital-based studies have shown reductions in skin flora and a reduction in the incidence of sepsis after topical chlorhexidine application. These studies involved term neonates predominantly.¹³

Chlorhexidine is well tolerated by term neonates when applied by various means of applications like vaginal washings, umbilical cord cleansing and whole body cleansing^{14,15,16}. Preterm infants less than 34 weeks of gestation, have immature skin with increased permeability. There is concern that the neurological system in preterms may be vulnerable to toxic insults. Preterm babies have metabolic limitations resulting in decreased drug clearance. These handicaps predispose them to a higher rate of adverse reactions from chlorhexidine.

Yet this population of preterm infants especially <34 weeks with increased susceptibility to infection and immature protective skin barrier may benefit from the antiseptics effects of chlorhexidine.

REVEIW OF LITERATURE

REVIEW OF LITERATURE

The mortality from neonatal sepsis in the very low birth weight (VLBW) and premature infant group has not changed much from 18-20% and 80% in the developed and developing world respectively for last three decades. The mortality is highest among these neonates and they stand twenty times greater chance of developing infection (often multiple) between birth and first month of life.¹⁷ There is also a greater chance of neuro-developmental delay among the neonates surviving the infections.¹⁸ Thus sepsis seems to be the most important cause of mortality and morbidity in this group of infants today. The sepsis rates are higher in these neonates because of increased exposure to microorganisms predisposed by risk factors and due to their weak host defense mechanisms.¹⁷

Risk factors predisposing to skin colonization

Newborn skin is virtually sterile and subsequent colonisation of the skin depends on varying exposures. Generally colonisation occurs within 2 - 7 days.¹⁹ By virtue of certain risk factors the infants are confronted with microorganisms from maternal or external environment.^{20, 21, 22}

Haque et al have identified 'risk factors' that predispose VLBW infants to skin colonization.²⁰

Maternal factors

The maternal risk factors that predispose to early skin colonization are repeated vaginal examinations in labour, presence of chorio-amnionitis, prolonged rupture of membranes (> 18 hours) and maternal urinary tract infection during pregnancy. The colonisation on the infantile skin is similar to the maternal habitat. A retrospective epidemiological study of neonatal infections acquired through maternal contamination was carried out in a maternity unit by Blond et al. The infection rate was 0.61% in newborns and 16% of the newborns, had asymptomatic colonisation by bacteria.²³ Among the various risk factors vaginal delivery and prolonged duration of premature rupture of membranes have significant positive correlation to neonatal colonization ($p < 0.02$ and $p = 0.02$, respectively) in the study by Ali GY et al.²⁴

Dermal factors

Acid mantle: The skin is alkaline at birth (pH of > 6). But in approximately four days an acid mantle develops (pH < 5). This acid mantle contributes for the protection against transcutaneous route of entry of microorganisms.²⁵

Vernix: Production of vernix begins by the end of 2nd trimester and most accumulate around 36-38 wks. The vernix caseosa helps to protect the fetal skin from damage from bacteria and amniotic fluid. It is composed of sloughed cells from the stratum corneum. It contributes to earlier skin acidification. In a study by Vissher et al in 2005, comparing term babies with retained vernix versus vernix devoid preterm infants at birth (pH– 5.16 vs. 5.97) and 24 hours (pH

4.9 vs. 5.63) at 24 hours is lower for vernix retained infants.²⁶ It is a natural skin cleanser and moisturiser. Vernix contains LL-37 and lysozymes that exhibit anti bacterial effects against pathogens such as E. Coli.²⁶ WHO guidelines recommends not to remove the vernix from newborn skin because of these anti- oxidant, anti- infective and wound healing properties.²⁷

Post natal events

Though microbial colonization begins immediately after birth, it is slow at onset and the rate increases after 12 hours.³ This process is expedited if they either require resuscitation at birth and or are admitted to neonatal units.

Other risk factors include low birth weight (1500 grams), GA less than 31 weeks, poor hand washing practices, umbilical catheterization, total parenteral nutrition (TPN), prolonged or un-necessary use of antibiotics and long line insertion.¹⁹ After birth, risk factors with highest significance were low birth weight, prematurity and use of invasive techniques ($p < 0.04$, $p = 0.03$ and $p = 0.03$ respectively).²⁴

The use of central venous catheters, mechanical ventilation, parenteral nutrition, and exposure to other invasive skin- or mucosa-breaching procedures in the nurseries increase the risk of CoNS infection substantially described by Dimitriou, G, et al. In that study, intubation and presence of central indwelling catheters were important risk factors for persistent CoNS infection when assessed separately; however, the biofilm production was the only significant effect when the risk factors were tested jointly (OR 4.69; 95% CI 1.59-13.84).²⁸

Weak defense mechanisms in Preterm neonates

The host defense mechanisms that are present in the newborns are

- Physical barriers – keratinised skin , mucus membranes enzymes and secretory IgA , etc.
- Passive immunity acquired through placenta
- Active immunity –both innate and adaptive or specific immunity.¹⁷

Skin – A weak barrier in Preterm infants

Maturation of the epidermal layers includes keratinisation, which results in the differentiation of granular and stratum corneal layers and the formation of a water-impermeable barrier. The stratum corneum of a preterm baby is thinner and immature than that of a term baby. Mature stratum corneum is made up of 10-20 layers of cells (a thickness of 2 mm) and the stratum corneum of preterm babies <30 weeks may only have 2-3 layers (0.9 mm thick). Thickness of the stratum corneum plays an important role in its barrier function. An intact mature stratum corneum helps to protect the skin from surface microorganisms.³⁰ (Fig.1)

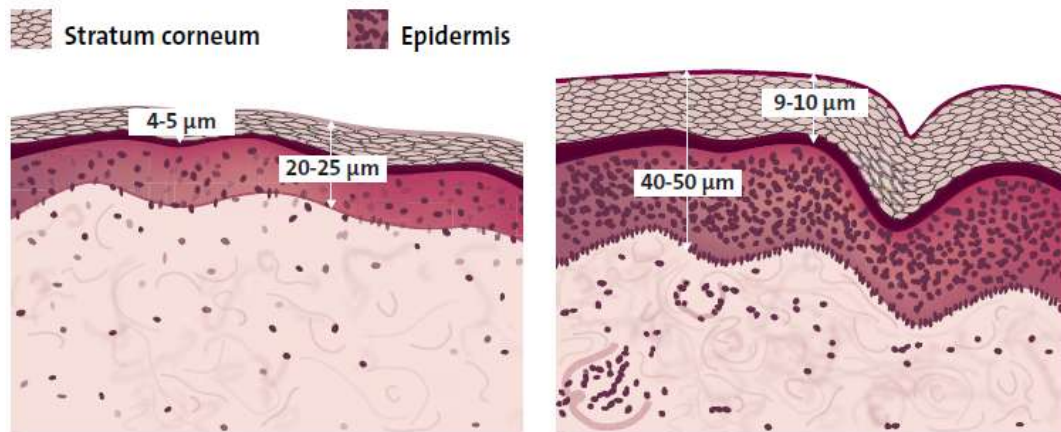


FIGURE : 1

Preterm skin at 26-27 weeks

Term infant skin

The stratum corneum begins to develop at approximately 24 weeks of gestation. The epidermis matures only around 32- 34 weeks of gestation. Before 32-34 weeks, the thin stratum corneum does not effectively prevent against invasion of microorganisms.⁵

The skin secretes Adenylate Mono Phosphate, which are early-response factors creating a microbicidal shield particularly effective against CoNS. In preterm neonates, the immature stratum corneum fully matures to secrete these factors only at one to two weeks after birth.³¹

Kalia et al studied the acid mantle in the preterm infants and stated that the acid mantle may not develop in preterm babies at the same rate as term neonates. In Premature infants pH is 5.5 after one week, 5.1 after one month, and in diapered area pH is 6.0 and hence their skin may not be well protected from bacteria.³²

The vernix caseosa, a waxy coating on neonates' skin mainly formed during the last trimester of pregnancy provides additional antimicrobial protection in mature neonates. Hence born too soon before 34 weeks deprives the preterm infants the mechanical barrier effect as well as the antimicrobial defense systems of the vernix.

Preterm infants have immature immune systems. Qualitative and quantitative deficiency of complement and IgG factors in these infants and particularly in the VLBW population increases the risk of CoNS infection. So even the normal flora may cause systemic infections in preterm infants because of the transcutaneous access through the immature skin barrier.⁶

Skin colonisation profile and transcutaneous sepsis

Neonates are normally colonized within 12 hours to first few days after birth by both Gram-negative and Gram-positive organisms and Candida species. Most common organisms to colonise among the flora are staphylococcus, streptococcus, acinetobacter, klebsiella and Candida species.^{3,33} Coagulase negative staphylococcus (CoNS) is a common inhabitant of the skin and mucous membranes as described by Hira et al. A small proportion of neonates acquire CoNS by vertical transmission, many acquire primarily horizontally. *S. epidermidis* was the most prevalent species among skin (33%), *S. warneri* (23% vs. 9%, $P=0.002$). *S. haemolyticus* prevalence increased significantly over time among skin isolates (9%, $T=24$ hours vs. 25%, $T=21$ days, $P=0.002$).³⁴

Several studies suggest that persistent CoNS infection is increasing in preterm infants in modern NICUs.^{28, 35} The rate of persistent CoNS infection ranges between 13% and 48%. CoNS is usually the causative pathogen in 40% to 77.6% of episodes of blood stream infection in neonates. The incidence is inversely related to gestational age and birth weight.³⁴

Chien et al. showed that 22.5% of infants admitted to NICU required CVCs and the incidence of blood stream infection in this study varies from 2.9 per 1000 non catheter days, to 7.2 per 1000 umbilical venous catheter days and 13.1 per 1000 percutaneous catheter days.³⁶

Protection against transcutaneous route

The options available for disinfecting the neonatal skin are povidone iodine, chlorhexidine and isopropyl alcohol.

Alcohol are rapidly bactericidal at 60-90% concentration rather than bacteriostatic. Alcohol causes skin burns in preterm neonates. They also evaporate rapidly, making extended exposure time difficult to achieve. FDA has not cleared any liquid chemical sterilant or high level disinfectant with alcohol as the main active ingredient.⁴⁵

Antimicrobial properties of iodine were first demonstrated in 1882 by Davaine. Povidone iodine has extensive evidence to support its use as skin disinfection before surgery and procedure. But its use is controversial in preterm newborns due to perceived issues with toxicity and systemic absorption.

Chlorhexidine – a better option

In the study by Mimos et al, the incidence of blood culture contamination was reduced more by chlorhexidine than povidone-iodine (14 of 1019 cultures [1.4%] compared with 34 of 1022 cultures [3.3%]; odds ratio, 0.40 [95% CI, 0.21 to 0.75]; $P = 0.004$).³⁷

A comprehensive review of current evidence in the Cochrane database found some evidence that skin preparation with 0.5% chlorhexidine in methylated spirits preoperatively was associated with lower rates of superficial skin infections following clean surgery than alcohol-based povidone iodine paint.³⁸

Linder et al described in two studies the effect of topical iodine-containing antiseptics in the preterm neonates on their thyroid function tests. The mean thyrotropin levels were elevated in preterm babies exposed to iodine (15.4 vs 7.8 mIU/L, $p < 0.01$). Among the iodine-exposed infants, elevated thyrotropin levels (> 30 mIU/L) were found in 13.7% of infants, compared with none in the chlorhexidine-treated group ($p < 0.01$). T4 and thyrotropin levels were measured weekly during the first 28 days, one every 2 weeks until the age of 60 days, and at the age of 90 days. Among iodine-exposed infants, 20.8% had thyrotropin values > 30 mIU/L, whereas none of the infants in the chlorhexidine group had elevated thyrotropin values ($p < 0.05$). Elevated urine iodine levels were present reflecting abnormally high iodine absorption.³⁹

Chlorhexidine

Mechanism of action

Chlorhexidine gluconate is used as a topical broad spectrum antiseptic. It is said to control antibiotic resistant bacteria and prevent infections. Chlorhexidine gluconate is used in concentrations ranging from 0.5% - 4% for topical antiseptic effect with or without alcohol.

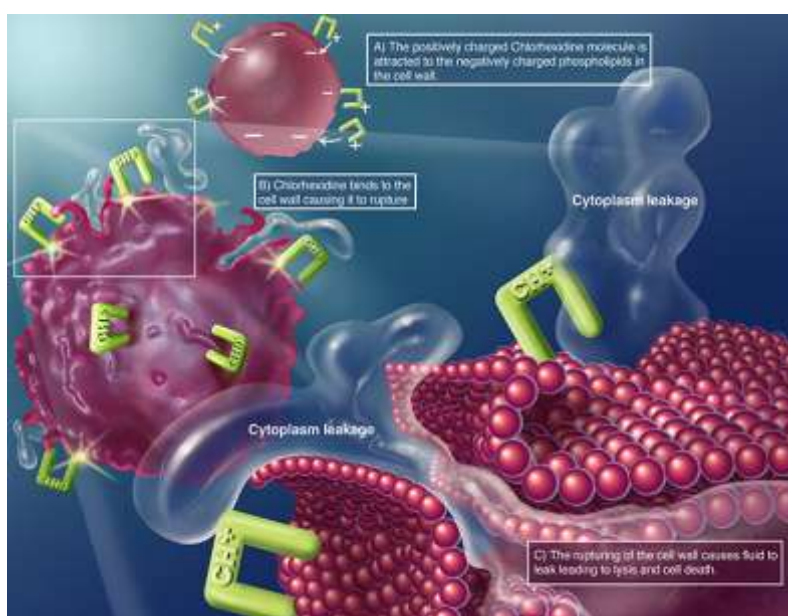


FIGURE 2 : MECHANISM OF ACTION OF CHLORHEXIDINE

In low concentrations it affects membrane integrity and high concentrations acts through cytoplasm causing cell death. Chlorhexidine is a positively-charged molecule. It binds to the negatively-charged sites on the cell wall and destabilizes the cell wall to interfere with osmosis. The bacterial uptake of the chlorhexidine is within 20 second.

The integrity of the cell wall is affected when applied in low concentrations. Once the cell wall is damaged, chlorhexidine then crosses into

the cell itself and attacks the cytoplasmic membrane (inner membrane). Damage to the cytoplasm's delicate semipermeable membrane allows for leakage of components leading to cell death. Chlorhexidine causes the cytoplasm to congeal or solidify in high concentrations.(Figure 2)

In topical applications, chlorhexidine has the unique ability to bind to the proteins present in human tissues such as skin and mucous membranes with limited systemic or bodily absorption. Chlorhexidine that is protein bound is released slowly leading to prolonged activity. This phenomenon is known as substantivity. This allows for a longer duration of antimicrobial action against a broad spectrum of bacteria and fungi. The antimicrobial activity of chlorhexidine has been documented to last 48 hours on the skin.⁴⁰ Chlorhexidine is used for the following purposes in the NICUs in United states⁴¹.(Figure 3).

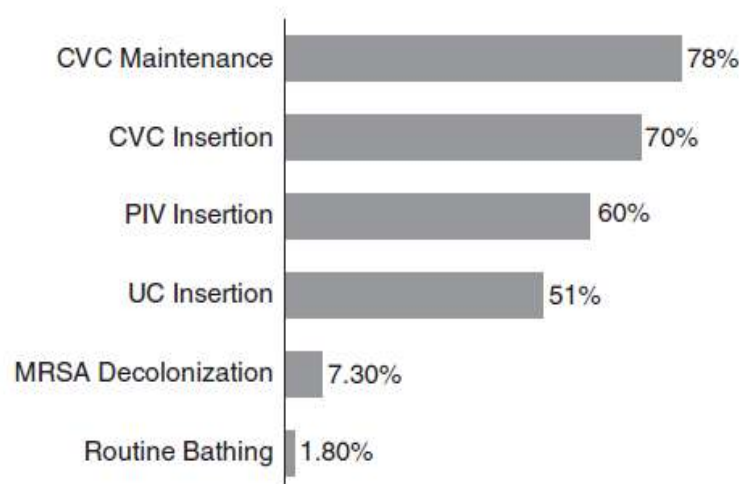


FIGURE 3 : USAGE OF CHLORHEXIDINE IN THE NICUs

Preparations

It is commercially available as

- 2 % CHG in 70% isopropyl alcohol
- 0.5 % CHG in 70 % isopropyl alcohol
- 2 % CHG aqueous

Literature from adult studies has shown that both 2% chlorhexidine in 70% alcohol and as 2% aqueous chlorhexidine can provide effective skin antisepsis though alcohol containing solution had more long lasting effect⁴². It is also well known from many case reports that alcohol containing products when used to clean abdominal skin for neonatal procedures can cause severe skin damage in preterm infants⁴³. As a result many neonatal units have adopted aqueous chlorhexidine as antiseptic agents.⁴³ Trials are planned and ongoing to compare the efficacy and safety of chlorhexidine in alcoholic and aqueous preparations.⁴⁴

Chlorhexidine is combined with alcohol for certain advantages. Alcohol has very rapid onset of action (10 seconds). When Chlorhexidine gluconate is in combination with alcohol the microbicidal action starts immediately on application to the surface. Moreover alcohol has got a good bactericidal action against gram negative organisms compared to chlorhexidine which has a high level of resistance to non fermenting gram negative organisms.⁴⁵

Adverse skin reactions

Chlorhexidine is used in the NICUs in US and a survey was conducted by Tammana et al on its usage. Fifty percent of the NICUs using chlorhexidine reported adverse skin reactions in that study but no NICU reported systemic toxicities. Skin burns was the most common reaction and erythema being second most common. These centers, which reported side effects noted that the burns occurred in neonates with birth weights <1500 g.⁴¹ Chlorhexidine with alcohol have been reported to cause burns in infants between 24 and 26 weeks gestational age compared to aqueous preparations.⁴⁶

Systemic absorption

Another issue of interest would be absorption of chlorhexidine through the immature skin of preterm infants. In spite of its recommendations for skin preparations before procedures, the Centre for Disease Control and Prevention is apprehensive about recommending chlorhexidine for infants less than 2 months of age⁴⁵. Much apprehension about chlorhexidine usage in NICUs is caused by the aftermaths created by its predecessor Hexachlorophene.

Hexachlorophene required multiple applications for its maximal antibacterial effects. But multiple applications more than 3 times produced irreversible brain damage in preterm causing vacuolar encephalopathy.⁴⁷

Serum levels of Chlorhexidine may correlate with the strength of the topical chlorhexidine solution used, as neonates exposed to 1% CHX had significantly higher blood concentrations of chlorhexidine compared with neonates exposed to 0.25% and 0.5% concentration solutions. Theoretically

sites like face, scalp may have increased vascularity contributing to increased blood levels when applied topically. However no site-specific safety data exists⁴⁸. Alcohol potentiates absorption of chlorhexidine when applied topically.⁴⁹ In general, the potential for absorption appears to be reduced when chlorhexidine is applied in aqueous or other non ethanol-based formulations⁵⁰.

Studies with chlorhexidine in neonates

Skin colonisation

The effect of first bath with chlorhexidine was studied by L.Da Cunha et al in a randomized masked trial in 2008 to reduce the staphylococcal colonization on newborn skin. The trial was conducted in ninety three neonates who received the first bath with chlorhexidine (n =44) or neutral liquid soap (n =49). *Staphylococcus aureus* colonization prevalence was 10.2% in control and 4.5% in the experimental group (p =0.74). Thirty minutes after bath, *S. Aureus* prevalence was 20.4% in control and 2.3% in the experimental group (p =0.017). Twenty four hours after bath, *S.Aureus* prevalence was 36.7% in control and 13.6% in the experimental group (p =0.021). There was no occurrence of sepsis in the first month in both groups. In conclusion, a first bath with chlorhexidine reduced *S.Aureus* colonization on the newborn's skin in a 24-h period.⁵¹

In a randomised controlled trial by Gary L Darmstedt et al in 2007, the skin of the hospitalized out born newborns admitted to a hospital, in Bangladesh was cleansed within 72 hours with baby wipes containing 0.25% chlorhexidine (n = 67) or placebo (n =66) solution. Skin swabs were taken from axillary, peri-

umbilical and inguinal sites at baseline and 2 hours, 24 hours, 3 days and 7 days after treatment. Skin colonization rates were analysed both qualitatively and quantitatively.

Percent of positive cultures at baseline varied by site: 74.4%, 39.1%, and 61.7% from the axillary, periumbilical, and inguinal sites, respectively. Skin colonisation rates at two hours after cleansing were approximately 35%–55% lower than the baseline rates for both groups at all three sites. For the chlorhexidine group, positive skin culture rates remained significantly lower than the baseline rates for 24 hours to three days, whereas for the placebo group, beyond the first 2-hour follow-up, these values were not lower than baseline in all 3 sites.

Chlorhexidine skin treatment produced more extended skin cleansing effects than placebo. The skin condition was not different between the groups. The reduction in the temperatures was also not significant. It was concluded that the possible quantitative and qualitative reductions observed in the skin flora might contribute to reducing neonatal infections.⁵²

In another randomized trial done by Luke C Mullany et al in 2008 among the hospital born newborns were randomly allocated to full-body skin cleansing with 0.25%, 0.5%, or 1.00% concentrations of chlorhexidine solution. Skin swabs were collected from the three sites - axilla, inguinal and peri-umbilical areas at baseline at 2hours and 24hours after treatment. The overall proportion of positive swabs at baseline was 60%. There was a dose-response trend for each of the separate sites. In all three study arms, the positive

skin culture rate was significantly lower at each of the three sites sampled 2 hours after the intervention. The reduction in colonization was greatest among the 1.00% group (63% reduction), followed by 0.50% group (50% reduction) and 0.25% group (48% reduction)⁴⁸.

At 24 hours, positive skin culture rates returned to baseline levels for all three sites in the 0.25% group. In the 0.50% group, only swabs from the axilla tended to still be lower than observed prior to the intervention, while in the 1.00% group, the positive rate remained lower than baseline at all three sites, and significantly lower among axillary and peri-umbilical swabs. Effect at 24 hours was highest in the 1.00% CHX group (37% lower positive skin culture rate); but did not achieve statistical significance.⁴⁸

In view of preterm population (<34 weeks) being less in number in the previous studies, MJ Sankar et al (2009) AIIMS randomised 28- 34 week infants within 3 hrs of birth into the following three groups: 0.25% chlorhexidine, normal saline or no skin cleansing. Skin condition, axillary temperature and skin colonization rates in the axilla and the groin were assessed at specified time intervals after intervention. Rate of culture positivity in the swabs taken at 24 hours were 22.2, 52.7 and 57.9% in the chlorhexidine, saline and no cleansing groups, respectively (P=0.06).

In the axillary region chlorhexidine reduced the risk of colonization by sixty two percent compared with no cleansing (RR: 0.38; 95% CI: 0.15, 0.98). However, no such reduction was observed when compared with saline (RR:

0.42; 95% CI: 0.16, 1.10). At 72 hours, the colonization was not different between the groups. In the groin no reduction in colonisation was observed either at 24hrs or 72 hrs after the cleansing. There was only a minimal reduction (mean 0.5°C) in body temperature and no adverse effects on skin condition were observed.⁵³

Mortality

The study conducted in Nepal, a community based trial by Teischl et al in 2007, cleansed newborn infants with infant wipes that contained 0.25% chlorhexidine soon after birth (median: 5.8 hours) and found reduction in neonatal mortality only among low birth weight infants.¹²

There were 4 more studies – 3RCTs^{12,14,54} and 1before and after study¹³ reporting on mortality after chlorhexidine intervention combining both vaginal washing and neonatal cleansing. After a pooled analysis in a systematic review there was no difference in mortality between the chlorhexidine and the control groups.⁵⁵

Reduction of sepsis

Da Cunha et al 2008, reported on decrease in staph colonisation rates in the neonates after chlorhexidine cleansing at birth. None of the neonates developed sepsis.⁵¹ The systematic review found no reduction in the incidence of sepsis.(RR0.97; 95% CI 0.80 – 1.18).⁵⁵

Hypothermia

Three studies (Pereira et al, Darmstadt et al, Sankar et al) reported hypothermia.^{16,52,53} Two studies reported greater incidence of hypothermia in the

chlorhexidine group in the first 30 minutes after the intervention.⁵² Majority of neonates had only mild hypothermia (36.0 to 36.4°C). But the study by Cunha et al showed no difference with chlorhexidine and neutral soap.

Skin reactions

Garland et al reported severe contact dermatitis with the use of a CHG-impregnated dressing that was placed over catheter sites after insertion⁵⁶. In these episodes of contact dermatitis secondary to CHX, it seems that the occlusive adhesive dressing causes external pressure restricting capillary perfusion to the skin and causing local skin breakdown.

There are no reports of contact dermatitis in neonates who received full-body skin cleansing with chlorhexidine, even in very low birth weight neonates and as young as 28 weeks gestational age. The trial at Zimbabwe in 2011 by L.Pereira et al¹⁶ has concluded that 1% CHX was safe in neonates. Two trials^{51,53} did not report any skin rash in both the control or the intervention group.

Systemic absorption of chlorhexidine

From the previous studies by Mullany et al, Aggett et al, Cowen et al^{48,49,50} comparing the blood concentrations of chlorhexidine was difficult because the samples were collected differently (heel stick versus venipuncture) and different laboratory assays were used for measurement. A standard method to detect CHX in the blood does not seem to exist. After cleansing with chlorhexidine, the percutaneous absorption that occurs, particularly in preterm neonates is only minimal. The strength of the chlorhexidine that is both effective and non-toxic when used topically is not known.

Gaps in knowledge and rationale for the present study

Preterms with their weak defense mechanisms would definitely benefit from the antiseptic effects of whole body cleansing with chlorhexidine. But there is paucity of evidence regarding the appropriate dose, dosing interval, clinical benefit and safety of chlorhexidine in this population. Previous trials with single application of 0.25% CHX were not producing consistent effects on reduction in skin flora. The previous alcoholic preparation of chlorhexidine produced considerable skin toxicity.

There is a need for a clinical trial with higher concentrations of chlorhexidine as multiple applications at intervals from birth to produce a consistent and sustained reduction effect on skin flora was felt.

The substantivity effect of chlorhexidine would extend only till 48 hours at the maximum. Hence multiple applications at this interval would be more appropriate.

But the safety of chlorhexidine when applied repeatedly on the immature preterm skin remains a concern in terms of skin toxicity, thermal stability and neurotoxicity.

The safety of aqueous form in the preterm skin without much increase in nosocomial colonisation and infection with gram negative organisms needs to be established.

HYPOTHESIS AND OBJECTIVES

HYPOTHESIS AND OBJECTIVES

Hypothesis

Multiple whole body cleansings with 0.5% chlorhexidine in haemodynamically stable neonates between 28 - 34 weeks of Gestational Age and birth weight more than 1000 grams will reduce the skin colonisation on the skin surface during the first weeks of life when compared to cleansing with sterile water.

Objectives

Primary Objective

To study the skin colonisation rates (swabs with culture positivity) on the seventh day of life in the axillary region after multiple whole body cleansings with 0.5% chlorhexidine (intervention group) and sterile water (placebo group) were measured and compared.

Secondary objectives: To compare the following in the infants after multiple whole body cleansings with either 0.5% chlorhexidine or sterile water.

1. Mortality within first 28 days of life.
2. Incidence of Sepsis within seven days of life
3. Proportion of infants requiring repeat hospital admissions within first 28 days of life.
4. Skin colonisation rates (swabs with culture positivity) at baseline, 24 hrs and 48 hrs after recruitment in the axilla and inguinal regions.

5. Mean bacterial colony counts in the culture positive swabs taken at baseline, 24 hrs, and 48 hrs after recruitment and on day seven of life in both the axillary and inguinal regions.
6. Mean skin temperatures at baseline, 5 and 15 mins after each application.
7. Skin condition by using the Newborn skin condition scoring scales. (Annexure - 6) before each cleansing and on seventh day.
8. Skin colonisation profile

MATERIALS AND METHODS

MATERIALS AND METHODS

Study design

Double blinded, Randomised controlled trial with a superiority framework.

Study centre

The study was conducted in the neonatal unit of the Institute of Obstetrics and Gynaecology, Madras Medical College, Chennai – a tertiary level Unit at Chennai.

Study Period

The study was undertaken from November 2013 to February 2014.

Subjects: Study population

Neonates between 28 -34 completed weeks of GA, admitted during the study period in the unit were assessed for eligibility with the following inclusion criteria

Inclusion criteria

- Hemo dynamically stable
- Birth weight more than 1000 grams
- No encephalopathy at the time of recruitment.

The following neonates were excluded

Exclusion Criteria

- Major congenital malformations
- Skin defects in epidermis involving more than 5% of body surface area.

Sample size

The sample size was determined from the pilot study undertaken in the unit as there were no studies documenting the effect of multiple applications of chlorhexidine on the skin colonisation.

The skin colonization rates in the Preterm infants on day 7 of life determined from the pilot study were 90% and 64.4% in the control and intervention group respectively. Thus to detect a difference of 25% between the intervention and the control groups using a two sided Fisher's Exact test with significance level of 0.05 and power of 90% we calculated a sample size of 60 infants in each group and enrolled 120 infants in the study .

Methodology

Assessment for eligibility

All Preterm infants between 28 – 34 weeks of completed gestation admitted during the study period within 6 hrs, weighing more than 1000 grams, haemodynamically stable and without encephalopathy were included in the study.

Gestational age (GA)

Gestational age was calculated by first trimester ultrasound dating or from the first day of the last menstrual period based on availability. Infants were classified based on the Fentons intrauterine growth charts. Infants whose birth weight fell below the 10th centile were considered as small for gestational and those weighing more than 90th centile as large for gestational age.

Hemodynamic stability was defined as follows:

1. Infants maintaining O₂ saturation of 88-92% without signs of respiratory distress either in
 - Room air or
 - Fio₂ requirement $\leq 50\%$, PEEP ≤ 7 cm of H₂O on CPAP
 - MAP ≤ 8 cm, Fio₂ $\leq 30\%$ on mechanical ventilation
2. Infants with normal perfusion status or requiring only one inotrope, dose not more than 10mcg/kg/min to maintain a normal perfusion status.

Infants with major congenital malformations and skin defects involving more than 5% of body surface area were excluded.

Consent and Ethical clearance

Informed written consent for the trial was sought from the parents before enrolment (Appendix 3A,4A). Parents were explained in detail about the relevance of the study and about the benefits and possible adverse reactions. They were also provided with the participation information sheet which was

printed in Tamil and English (Appendix 3B,4B). The trial was cleared by the Institutional Ethical Committee. (Annexure 1A - EC Reg No. ECR/270/Inst./TN/2013).

Stratification

Neonates were stratified into two groups based on the gestational age; Stratum A (28-31 weeks) and stratum B (32-34 weeks). Within each stratum, infants were randomised to receive cleansing with either 0.5% chlorhexidine (intervention) or sterile water (placebo).

Randomisation

Computer generated random numbers with variable block sizes (4 to 6) were used to allocate the neonates into the intervention and the control groups.

Zipped packets containing 4 individually sealed wipes were numbered according to the computer generated random numbers. (Fig.5) Packets were opened in the serial order after recruitment of each patient and wipes from that packet were used for three cleansings performed at 0, 48 and 96 hours after recruitment. (Fig.6) Fourth wipe was placed in the packet for an eventuality of accidental tumbling.

Blinding

The packets containing the wipes with either chlorhexidine or sterile water were prepared by the same manufacturer and they looked similar in all respects. Neither the care giver nor the principal investigator could make out any difference between the wipes



FIGURE: 4. WIPES IN SEALED COVERS



FIGURE: 5. FOUR WIPES IN ZIPPED PACKETS



FIGURE: 6. PACKETS ARRANGED IN SERIAL ORDER

Procedure

After recruitment, the baseline characteristics were entered onto the data collection forms (Annexure 2). The skin condition of the infant was scored according to the Newborn skin condition scoring scales (Annexure 6) and the axillary temperature was recorded before cleansing, at 5min & 15 min after each cleansing procedure.

Intervention

The neonates were cleansed with specially prepared body wipes sealed in covers and packed in zipped packets. Each contained four sealed wipes. Inside the sealed cover there was a single wipe which was divided into five portions by an impression line (Fig.4 & 8).

The duty staff/principal investigator wiped the infant skin (except face and scalp) from the neck till the soles in five steps. The body was divided into five parts and one portion of the wipe was used for each part of the body .The parts were; Neck and chest, Abdomen, Perineum, Upper limbs and Lower limbs (Fig.9).

To achieve consistency in the procedure of skin cleansing, the nurses in the nursery were trained using wipes on a mannequin. Instruction sheet was made available in the unit during the study period.



FIGURE: 7. SKIN SMEAR TAKEN FROM AXILLA



FIGURE: 8. CHLORHEXIDINE AND STERILE WATER WIPES



FIGURE: 9. CLEANSING PROCEDURE

Physician in charge took decision about the day to day management of the neonate. He / she were not a part of the study and were blinded to the intervention. Routine skin care was not provided during the period of study. The infants were followed up for development of adverse reactions on the skin and for sepsis.

Skin smears

Skin smears were taken with sterile swabs from the right axilla and the right inguinal regions at baseline, 24 hrs and 48 hrs after recruitment and on the 7th day of life (Fig.7). Swabs were pre-soaked in distilled water. Swabs were rubbed five times both horizontally and vertically rotating 360 degrees in the sampling area. The swabs were placed in transport medium (Trypticase soy broth) and sent to the micro lab immediately after collection (Fig.11 & 12). To ensure proper dispersion of the contents of the swab, they were vortexed into the medium, then the inoculum (0.01 ml) was placed onto the sheep blood agar and Mac Conkey agar media by using a calibrated loop and then incubated at 37 °C for 18 to 24 h.



FIGURE: 10. SWABS AFTER COLLECTION

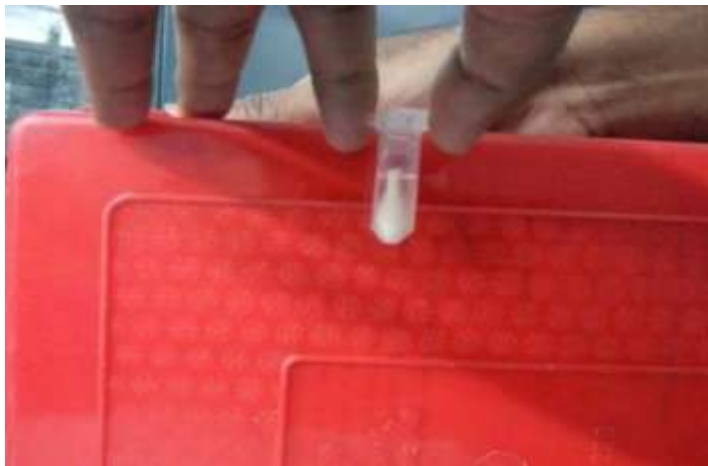


FIGURE: 11. SWABS TRANSPORTED IN TRYPTICASE – SOY BROTH

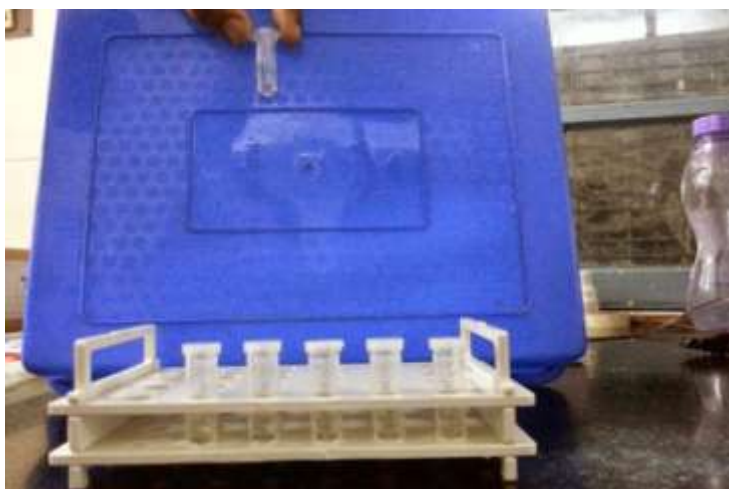


FIGURE: 12. SWABS VORTEXED IN MEDIUM

Skin colonisation

Skin colonisation was measured by

Qualitative analysis: culture positive swabs (growth of any organism)

Gram staining was done as per the standard procedure.

Quantitative analysis: bacterial colony counts from the swabs showing positive growth.

Bacterial colony counts were determined by semi quantitative analysis (100 colonies in 0.01 ml inoculums would amount to 10^5 colonies per milliliter).

Sepsis work up

The infants were subjected to detailed sepsis work up on clinical suspicion of sepsis until 7 days of life.

1. Sepsis was defined based on combination of clinical course, indirect laboratory markers and bacterial culture results

2. Sepsis defined as:

Culture positive: Infants with signs and symptoms suggestive of sepsis and blood culture positive.

Culture negative: Infants with signs and symptoms suggestive of sepsis, positive sepsis screen and blood culture negative.

3. Positive sepsis screen : ≥ 2 parameters positive

Total leucocytes count $<5000\text{mm}^3$, ANC high or low as per mouzinho's charts , micro ESR (1 hour $>$ age +3 mm in first 7 days of life) and qualitative CRP.

Follow up

All infants were followed up until 28 days of life prospectively during the routine postnatal checkups and immunisation sessions and through telephonic contacts for readmissions and neonatal mortality.

Data collection: All the data were entered in the data collection form.

(Annexure 2)

Statistical analysis

Categorical variables are reported in proportions (odds ratio or relative risk with 95% C.I) Chi square was used for comparison. Continuous variables are reported with mean or median and standard deviations or inter quartile ranges. Student t test was used for comparison when the data had a normal distribution and non parametric tests like Mann Whitney U tests and Wilcoxon rank sum analysis were used for skewed distribution. A P value < 0.05 was considered statistically significant.

FIGURE 13

TIME LINE OF EVENTS DURING THE STUDY

DAY OF LIFE



 SKIN SCORING

Measurement of outcomes

Table 3.1 Measurement of outcomes in the study

Outcome	Time	Tools	Sampling	Reporting
Skin colonisation <ul style="list-style-type: none"> • Culture positive swabs • Colony counts 	<ul style="list-style-type: none"> • Baseline • 24 hrs • 48 hrs after 1st cleansing • Day 7 	Sterile swabs Trypticase soy broths Sheep blood agar Macconkey agar	Principal investigator	Microbiologist
Skin temperatures	<ul style="list-style-type: none"> • Baseline • 5min • 15min after each cleansing 	Temperature probe Digital thermometer		Principal investigator
Skin condition	<ul style="list-style-type: none"> • Baseline • before each cleansing • Day 7 	Newborn skin condition scoring scales		Principal investigator
Sepsis workup	On clinical suspicion	Total leucocytes count Absolute neutrophil count CRP μ - ESR	Primary physician	Microbiologist Pathologist
Follow-up	Day 28 of life	PN visits Telephone contacts PN visits		Principal investigator

RESULTS AND ANALYSIS

RESULTS

During the study period 154 neonates between 28-34 weeks of gestational age were born in our Institute. Of these 137 were eligible for inclusion. Of them 120 neonates were recruited for the study after obtaining parental consent. The included neonates were 28-34 weeks of gestational age neonates who were admitted into the unit, who weighed more than 1000 grams, and stable haemodynamically without encephalopathy. 17 preterm infants were excluded for reasons like skin defects involving more than 5% of Body surface area(1), major congenital malformations(4), delay in recruitment (9) and non availability of the principle investigator (3).

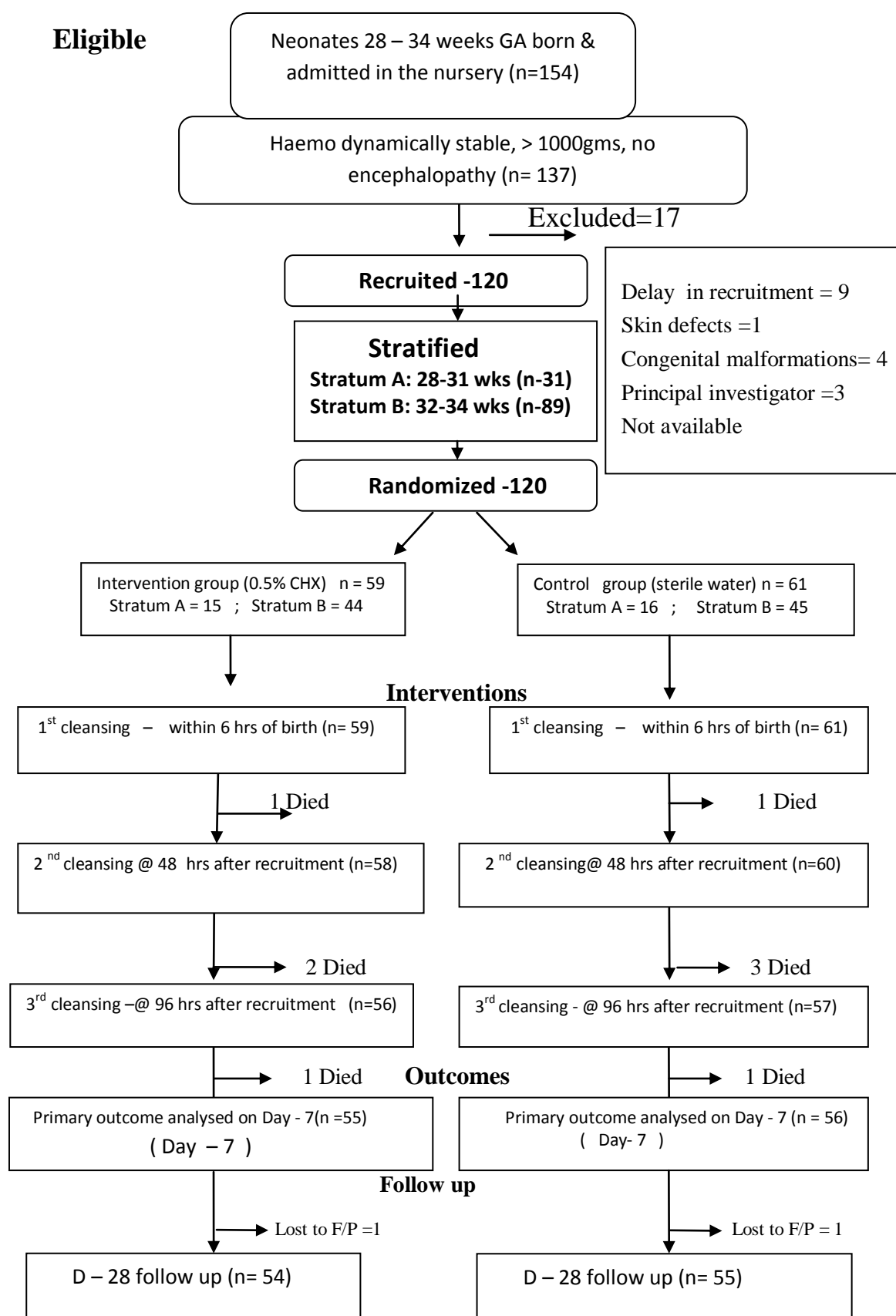
120 preterm neonates who were enrolled were stratified into two strata. Stratum A (28-31 weeks gestation) had 31 neonates and stratum B (32-34 weeks gestation) had 89 neonates. The stratified neonates were randomised by the computer generated randomised sequence into either the intervention group (n=59) or the control group (n=61). The intervention group received whole body cleansing with wipes containing 0.5% chlorhexidine gluconate and the placebo group cleansing with wipes containing sterile water. The baseline neonatal and maternal characteristics were collected and tabulated.

All of the 59 neonates who were allocated to the chlorhexidine group, 59 infants received the intervention within 6 hrs of birth. Since one infant died on the second day and was not available for intervention at 48 hours 58 infants were cleansed at 48 hours after recruitment. Subsequently 2 infants died and only 56 neonates received the third cleansing at 96 hours after recruitment.

Similarly in the placebo group 61 infants received the first cleansing at recruitment, 60 infants at 48 hours and 57 infants at 96 hours after the recruitment. Totally the intervention and the control groups had four and five deaths respectively. The chlorhexidine group had 55 infants and the placebo group had 56 infants for analysis of the primary outcome on day 7 postnatal age. The infants were monitored until the end of first week of life for features of sepsis as per the unit protocol.

The infants were followed up after discharge until day 28 during their visits for routine post-natal checkups and immunisations or by contacting through telephones if they had not been brought for follow up. The details in case of mortality and repeat hospital admissions were sought. Two infants one in intervention and one in control group were lost to follow-up after discharge from the hospital (Fig 14).

FIGURE 14. RANDOMISATION FLOW CHART



COMPARISON OF BASELINE CHARACTERISTICS

Table 4.1: Maternal characteristics

Variable	Chlorhexidine (N= 59) n (%)	Sterile water (N=61) n (%)	P value
Maternal Age (yrs)¶	25 ± (4.5)	24.6 ±(4.4)	0.631
Literacy rate	27 (45.8)	31 (50.9)	0.710
No.of ANC visits	3.7 (1.1)	4.0 (1.2)	0.199
AN steroids coverage			0.574
Nil	27 (45.8)	34 (62.3)	
Partial	12 (20.3)	10(16.4)	
Complete	20 (33.9)	17(27.9)	
Mode of delivery			0.099
Normal vaginal	30 (50.8)	24 (39.3)	
Instrumental	8 (13.6)	4 (6.6)	
LSCS	21 (35.6)	33 (54.1)	
ROM duration(hrs) (median, IQR)	8 (4 ; 25)	17.5 (6 ; 44.3)	0.296
Maternal infections	4 (6.8)	2 (3.3)	0.645
mothers administered Intra partum antibiotic prophylaxis	13 (22)	12 (19.7)	0.925

¶- mean (S.D), AN – Antenatal, ROM – Rupture of Membranes

Maternal characteristics like age, educational status, antenatal care, mode of delivery, underlying maternal infection were comparable between the groups. Mothers who were covered with antenatal steroids and intrapartum antibiotic prophylaxis were distributed equally between the groups. Mothers with fever, urinary tract infections and chorioamnionitis were comparable in numbers in both the groups.

Table 4.2: Maternal Socio- economic status

Socio- economic status	Chlorhexidine (N= 59) n (%)	Sterile water (N=61) n (%)	p- value
Upper (I)	0 (0)	0 (0)	0.011
Upper middle (II)	14 (23.7)	26 (42.6)	
Lower middle (III)	33 (55.9)	32 (52.5)	
Upper lower (IV)	12 (20.3)	3 (4.9)	
Lower (V)	0 (0)	0 (0)	

Among the demographic characteristics, the socioeconomic status of the mothers scored using the modified Kuppuswamy scale was different between the two groups.

More number of mothers of the infants who were randomly allocated to the intervention (chlorhexidine) group belonged to the lower socioeconomic class than the mothers of the infants allocated to the placebo group. Difference in distribution was statistically significant (p = 0.011) across the five classes (Table 4.2).

Cause for Preterm delivery

The most common cause for the preterm delivery was spontaneous onset of preterm labour without any precipitating factors. Preterm deliveries due to preterm premature rupture of membranes were almost of equal incidence in both the groups. (Table 4.3)

Table 4.3 Causes for Preterm delivery

Cause of Preterm delivery	Chlorhexidine (N= 59)	Sterile water (N=61)	p- value
	n (%)	n (%)	
Spontaneous labour	28 (47.5)	30 (49.2)	0.943
pPROM	9 (15.3)	8 (13.1)	
Indicated	22 (37.3)	23 (37.7)	

Neonatal Characteristics

The groups were comparable in terms of mean gestational age, birth weights and postnatal age at recruitment. Infants born out of multiple pregnancies were equal in proportions in both the groups. Not many preterm infants had much vernix distribution on their skin (71.2 % of them with less than 25 % distribution) and proportion was similar in both the groups. The proportion of preterm babies requiring resuscitation at birth were comparable in the intervention and the control groups respectively (Table 4.4).

Table: 4.4 Baseline neonatal characteristics.

Variable	Chlorhexidine (N= 59) n (%)	Sterile water (N=61) n (%)	P value
Gestational Age wks ¶	32.5 (1.8)	32.5 (1.8)	0.875
Birth weight (gms)¶	1691.7 (403.7)	1766 (425.7)	0.325
Age at recruitment (mins)¶	197.7 (96.4)	167.8 (98.8)	0.096
Males	25 (42.5)	33 (54.1)	0.270
Multiple births	17 (28.8)	12 (19.7)	0.339
Vernix < 25 (%)	42 (71.2)	36 (59.0)	0.228
25 – 50 (%)	17 (28.8)	25 (41.0)	
Neonates resuscitated at birth	12 (20.3)	10 (16.4)	0.598

¶- mean (S.D)

Table 4.5: Distribution of infants across the gestational ages

Distribution of gestational age	Chlorhexidine		Sterile water		P- value
	n	%	n	%	
28 – 30 wks	8	(13.6)	9	(14.8)	0.913
31 – 32 wks	13	(22.0)	15	(24.6)	
33 – 34 wks	38	(64.4)	37	(60.7)	

The distribution of infants across different gestations among the intervention and the control groups was similar. More mature infants predominated in both the groups. (64.4% Vs 60.7) (Table 4.5)

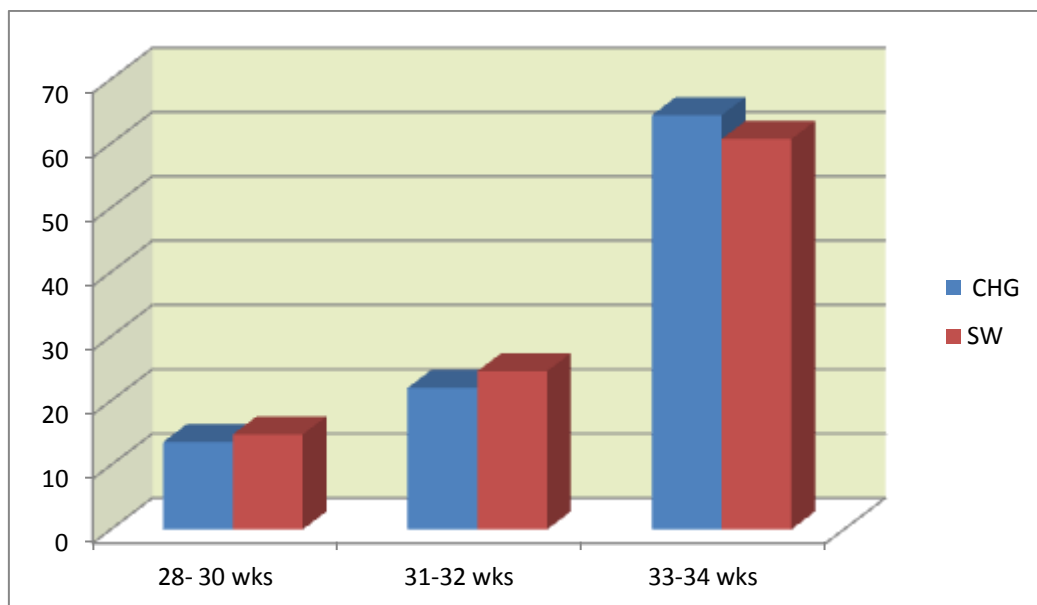


Figure 15. Distribution of infants across the gestational ages

Table 4.6: Distribution of birth weight of infants

Distribution of Birth weight (gm)	Chlorhexidine n %	Sterile water n %	P- value
1000 – 1499	21 (35.6)	17 (27.9)	0.290
1500 – 1999	26 (44.1)	24 (39.3)	
2000– 2499	11 (18.6)	15 (24.6)	
≥ 2500	1 (1.7)	5 (8.2)	

Both the study groups had comparable distribution of birth weight groups. Larger neonates were more in the placebo group but was statistically insignificant ($p=0.290$) (Table 4.6).

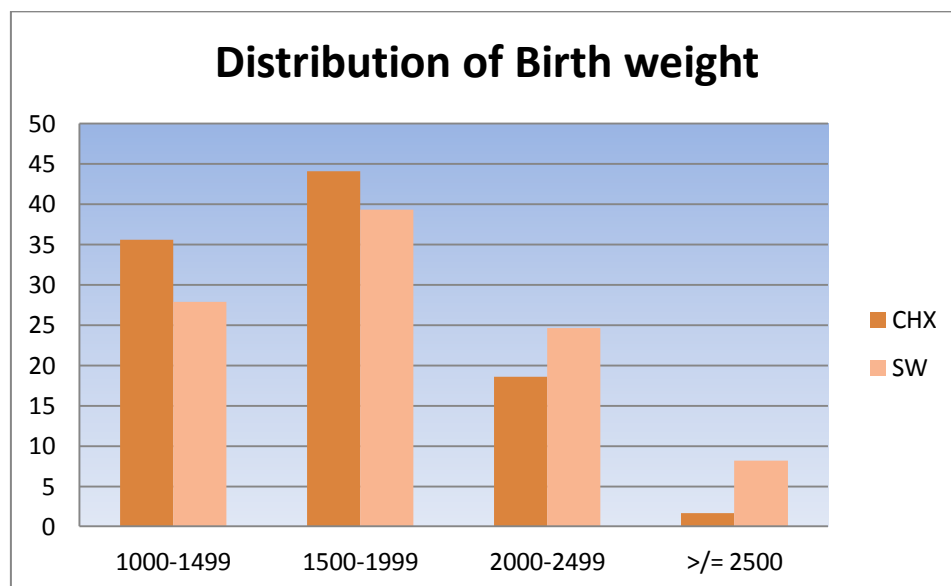


Figure 16. Distribution of birth weight

Table 4.7: Intrauterine growth status of the infants

Intrauterine growth status	Chlorhexidine n %	Sterile water n %	P- value
AGA	42 (71.2)	47 (77.0)	0.588
SGA	15 (25.4)	11 (18.0)	
LGA	2 (3.4)	3 (4.9)	

AGA- appropriate for gestation

SGA- small for gestation

LGA- large for gestation.

The intrauterine growth status of the preterm infants was comparable in both the groups. Majority of them were appropriate for gestational age (Table 4.7)

Table 4.8: Postnatal risk factors for skin colonisation

Variable	Chlorhexidine (N=59) n (%)	Sterile water (N=61) n (%)	P value
Mechanical ventilation	16 (27.1)	24 (39.3)	0.220
Ionotropic support	10 (17.2)	15 (24.6)	0.420
Intravenous access	36 (61.0)	43 (70.5)	0.812
IV access duration ¶	8 (5- 15.6)	7 (5 ; 15)	0.774
PICC line insertion	12 (20.3)	12 (19.6)	0.891
PICC line duration ¶	6.5 (5 - 8)	6.5 (5 ; 8)	0.887
Umbilical line access	3 (5.08)	4 (6.55)	0.963
UVC line duration ¶	4 (3- 5)	4 (3.2-5.5)	0.857
Surfactant therapy	10 (16.9)	17 (27.9)	0.225

¶ -Median (interquartile ranges) in days

The postnatal risk factors which predispose to skin colonisation like mechanical ventilation, ionotropic support, venous access through central and peripheral lines and surfactant therapy were comparable in both the groups. ($p > 0.05$) (Table 4.8).

Primary outcome variable

Skin colonisation rate in the axilla after the intervention

Table 4.9 : Positive skin culture rates in the axilla

Time	Chlorhexidine n (%)	Placebo n (%)	RR (95% C.I)	P value
Day – 7	22/55 (40.0)	45/56 (80.3)	0.50 (0.44-0.56)	<0.001

The skin colonisation rates after the intervention on day-7 of life were 40.0% and 80.3% in the chlorhexidine and sterile water groups respectively. The reduction in skin colonization was statistically significant ($P < 0.01$). The risk of colonization was reduced in the chlorhexidine group by 50% after multiple cleansings as compared to sterile water cleansing. The absolute risk reduction was 40.3%. Thus the number needed to treat was 2 persons. (NNT=2) (Table 4.9).

Secondary outcome variables

Skin Colonisation during various time lines.

Table 4.10 : Positive skin culture rates in axilla during 48 hrs

Time	Chlorhexidine n (%)	Placebo n (%)	RR (95% C.I)	P value
Baseline	38/59 (64.4)	27/61 (44.3)	1.45 (1.15, 1.74)	0.027
24 hrs	37/58 (63.8)	43/57 (75.4)	0.85 (0.77, 0.93)	0.175
48 hrs	31/55 (56.4)	49/58 (84.5)	0.67 (0.59, 0.75)	<0.001

The skin colonisation rate at axilla was higher at the baseline in the chlorhexidine group by 20.1% than the placebo group. This difference was statistically significant. Colonisation rates were higher in the placebo group thereafter. The difference in colonization observed among the groups at 48 hours was significant ($p<0.001$). Even after single cleansing chlorhexidine group showed a reduction of 28.1% in the colonisation rates. The risk of skin colonization at the axilla was reduced by 33% in the chlorhexidine group at 48 hours after a single cleansing. (Table 4.10).

Table 4.11: Positive skin culture rates in the groin

Time	Chlorhexidine n (%)	Placebo n (%)	RR (95% C.I)	P value
Baseline	35/59 (59.3)	21/ 61 (34.4)	1.72 (1.23, 2.21)	0.006
24 hrs	33/58 (56.9)	44/57 (77.1)	0.74 (0.66 ,0.82)	0.021
48 hrs	34/55 (61.8)	50/58 (86.2)	0.72 (0.64 ,0.81)	0.002
Day – 7	28/55 (50.9)	46/56 (82.1)	0.62 (0.55, 0.71)	<0.001

The skin colonisation rate after the intervention on day-7 of life was 50.9% and 82.1% in the chlorhexidine and sterile water groups respectively. The difference in the skin colonization rates was statistically significant ($P < 0.01$). The absolute risk reduction was 31.2%. Thus the risk of colonisation in the groin was reduced by 38% on the seventh day of life after repeated chlorhexidine cleansing. The number needed to treat with this reduction was 3 persons at the groin (NNT=3). The colonisation rates in the groin in the intervention group at 24hours, 48hours and on day 7 were all significantly lower than the placebo group in the groin. (Table 4.11)

The skin colonisation rates in the groin remained higher than the axilla during the study period except at baseline.

Bacterial colony counts in the culture positive swabs

Table 4.12 : Colony counts in the axilla during the study period

Time	Chlorhexidine		Placebo		P value
	n	median (IQR)	n	median (IQR)	
Baseline	59	10^2 (0 – 10^3)	61	0 (0 – 10^3)	0.098
24 hrs	58	$10^{2.7}$ (0 – 10^4)	57	10^3 ($10^{1.7}$ - 10^4)	0.351
48 hrs	55	10^2 (0 – 10^4)	58	10^3 (10^2 - 10^4)	0.011
Day 7	55	0 (0- 10^3)	56	10^4 (10^2 - 10^5)	< 0.001

Table 4.13 : Reduction in the colony counts in the axilla

Time	Chlorhexidine		Placebo		p- value
	n	median (IQR)	n	median (IQR)	
0 – 24 hrs	58	0(10^4 to -10^2)	57	10^2 (10^4 to 0)	0.164
24-48 hrs	55	0(10^3 to -10^3)	55	0 (10^3 to -10^2)	0.366
48 hrs – D7	53	0(0 to -10^3)	53	0 (10^5 to $-10^{2.7}$)	0.006
0 – D7	53	0(0 to -10^3)	52	10^3 (10^5 to 0)	<0.001

The median colony counts taken at 48 hrs (10^2 and 10^3 , $p=0.006$) and on the seventh day of life (0 and 10^4 , $p<0.001$) from the axilla were significantly less in the chlorhexidine group than the sterile water group.(Table 4.12). The quantitative reduction from the baseline on day – 7 of life was significant between the groups. (Table 4.13).

Table 4.14 : Colony counts in the groin during the study period

Time	n	Chlorhexidine median (IQR)	n	Placebo median (IQR)	P value
Baseline	59	10^2 (0 to 10^3)	61	0 (0 - 10^3)	0.041
24 hrs	58	10^2 (0 - 10^4)	57	10^3 (10^2 - 10^4)	0.042
48 hrs	55	10^2 (0 - 10^4)	58	10^4 (10^2 - 10^5)	0.004
Day-7	55	$10^{1.7}$ (0 - $10^{3.9}$)	56	10^4 (10^2 - 10^5)	0.000

The median colony counts in the chlorhexidine group were significantly lower than that of sterile water at 24 hours (10^2 and 10^3 , $p=0.042$), 48 hours (10^2 and 10^4 , $p=0.004$) and on the seventh day of life ($10^{1.7}$ and 10^4 , $p<0.001$) in the groin. (Table 4.14)

Table 4.15 : Reductions in colony counts in the groin during the study period

Groin	Chlorhexidine		Placebo		p-value
	n	median (IQR)	n	median (IQR)	
0 - 24 hrs	58	0 ($10^{2.6}$ to -10^2)	57	10^2 (10^4 to 0)	0.030
24 - 48 hrs	55	10^4 (0 to -10^5)	55	0 (10^4 to 0)	0.094
48 hrs – D7	53	0 (0 to -10^3)	53	0 (-10^3 to $-10^{3.7}$)	0.107
0 - Day 7	53	0 (10^3 to -10^3)	52	10^3 (10^5 to 0)	0.000

The reduction in the colony counts at 24 hrs (0 and 10^2 , P= 0.030) and day seven (0 and 10^3 , P<0.001) from the baseline were significant in the chlorhexidine group as compared to the placebo group. (Table 4.15)

Table 4.16 : Outcomes on Follow up

Status on D- 28	Chlorhexidine (N=58) n (%)	Sterile water (N=60) n (%)	P value
Mortality	6 (10.1)	10 (16.3)	0.451
Discharged	44 (74.5)	42 (68.8)	0.621
Sick in hospital	0 (0)	7 (11.7)	0.007
Repeat hospitalisation	0 (0)	6 (14.6)	0.029

There was no significant difference in mortality rates between the infants in the chlorhexidine and the sterile water groups. The discharge rates among both the groups were similar. There were 8 infants in the chlorhexidine group who remained in the hospital for weight gain. The number of infants who were sick on Day 28 and the proportion that required repeat hospitalisation were significantly lower in the intervention group. (Table 4.6).

Table 4.17 : Incidence of sepsis during the first week

Gestational age weeks	N	Chlorhexidine n (%)	N	Sterile water n (%)	P value
28 - 34	59	17 (28.8)	61	23 (37.7)	0.401
28 - 31	15	6 (40.0)	16	12 (75.0)	0.048
32 - 34	44	11 (25.0)	45	11 (24.4)	0.952

Out of 120 neonates 59 underwent septic workup. 18 patients had culture positivity (30.5%) and 22 infants had septic screen positive or clinical course suggestive of septicaemia.

Two infants in the sterile water group had meningitis and none in the chlorhexidine group. 4 cases of skin infection were (IV site abscess) observed in the sterile water group. Though overall sepsis rates were not different between the two groups, on subgroup analysis less mature infants (28 – 31 weeks GA) in the placebo group had higher incidence of sepsis than their counterparts in the chlorhexidine group. (Table 4.17).

Table 4.18 : Mean skin temperatures during cleansing

Cleansing	Time (min)	Chlorhexidine mean (S.D)	Sterile water mean (S.D)	P- value
First	0	36.5(0.1)	36.5 (0.1)	1.000
	5	35.7(0.8)	35.9 (0.5)	0.281
	15	36.5(0.1)	36.5 (0.1)	0.501
Second	0	36.4 (0.4	36.4 (0.2)	0.890
	5	35.9(0.6)	36.0 (0.5)	0.322
	15	36.4 (0.3)	36.4(0.3)	0.762
Third	0	36.4 (0.3)	36.3 (0.3)	0.440
	5	36.1 (0.5)	36.0 (0.5)	0.583
	15	36.4 (0.3)	36.3 (0.3)	0.928

Mean skin temperatures at baseline, 5 and 15 minutes after all the three cleansing procedures were comparable between the two groups. Though there were variations in the mean temperatures within the groups it was comparable between the groups in both the sub groups. (Table 4.18).

Table 4.19 : Incidence of cold stress and hypothermia during cleansing

Wipe	Time (mins)	Chlorhexidine n (%)	Placebo n (%)	P value
First	0	0 (0)	0 (0)	-
	5	27 (45.8)	26 (42.6)	0.87
	15	0 (0)	1 (1.6)	1.000
Second	0	2 (3.5)	2 (3.3)	1.000
	5	22 (37.9)	21 (35.0)	0.889
	15	3 (7.4)	5 (8.3)	1.000
Third	0	3 (5.4)	1 (8.8)	0.733
	5	17 (30.4)	18 (31.6)	1.000
	15	3 (5.4)	4 (7.0)	1.000

The proportion of the patients who slipped into the cold stress and hypothermia gradually decreased as the postnatal age advanced. There was no significant difference in proportion of patients who experienced cold stress and hypothermia between the two groups (Table 4.19).

Comparison of skin condition scores

The skin condition of the infants cleansed with chlorhexidine were not different from that of the infants cleansed with sterile water. Skin changes like erythema, drying, scaling or fissuring were not observed in any of the infants in both the groups.

Table 4.20 : Gram Negative organism colonisation

Site / time	Chlorhexidine		Placebo		P- value
	n	%	n	%	
Axilla-baseline	17	(44.7)	9	(33.3)	0.355
Axilla -24 hrs	22	(59.5)	29	(67.4)	0.459
Axilla- 48hrs	18	(58.1)	35	(71.4)	0.218
Axilla- day 7	9	(40.9)	23	(51.1)	0.432
Groin-baseline	11	(31.4)	9	(42.9)	0.405
Groin -24 hrs	19	(57.6)	33	(75.0)	0.106
Groin - 48hrs	24	(70.6)	32	(64.0)	0.530
Groin- Day 7	17	(60.7)	25	(54.3)	0.592

Colonisation with gram negative pathogens were comparable in both the groups (Table 4.20).

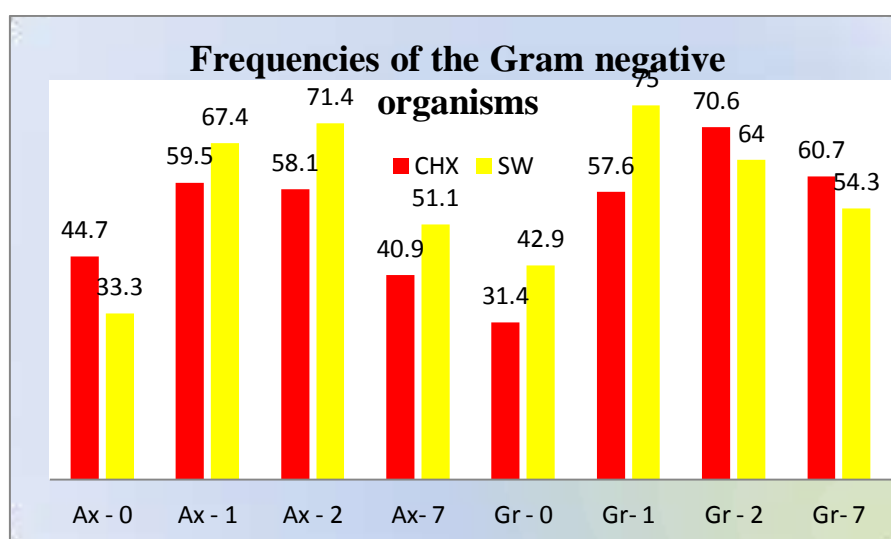


Figure 17 : Frequencies of the Gram Negative organisms

DISCUSSION

DISCUSSION

Characteristics of the study population

In our study we enrolled 120 haemo dynamically stable preterm infants whose mean gestational age and birth weight were 32.5 (± 1.8) weeks and 1700 (± 403) grams comparable in both the intervention and placebo groups. No sex predominance was observed. Majority of them were recruited at a mean postnatal age of 3 hours.

The study population in the previous trials^{12,48,52} were dominated by the mature term neonates and their conclusions on efficacy and safety profile of chlorhexidine in neonatal medicine may not be applicable to the vulnerable preterm population. Hence M.J Sankar et al⁵³ studied the preterm population exclusively enrolling 28-36 weeks GA infants weighing 1000-2000 grams at birth. They sampled a relatively small number of preterm infants (20 infants in the intervention and 20 in control groups).

The baseline parameters of the mothers were also comparable. The proportion of infants delivered by vaginal route was 53%. All but one (the socioeconomic status of the mothers classified according to the modified Kuppuswamy classification, (Annexure 5) were comparable between the groups. The socioeconomic status of the mothers showed a significant difference. Previous studies did not include the socioeconomic status in the demographic profile. We speculate that this would have a bearing on the maternal vaginal colonisation due to the poor hygienic status prevailing in the lower socioeconomic class.

The postnatal risk factors which favour skin colonisation like respiratory and inotropic support, surfactant therapy and venous access through peripheral and central lines were comparable between the groups.

Skin colonisation

In our study, multiple whole body cleansings with 0.5% chlorhexidine showed significant reduction in the skin colonisation rates on day seven of life in the axillary and groin regions as compared to cleansing with sterile water. The risk of colonisation was reduced in the axilla by 50% and groin by 38% with three applications of 0.5% chlorhexidine. Higher percentage of reduction was produced by chlorhexidine in the axilla than in the groin.

Not many studies had looked at skin colonisation at the end of the first week but for the one conducted by Gary Darmstedt et al ⁵² among the out born hospitalised neonates. This study evaluated the impact of a single application 0.25% chlorhexidine. This trial could not achieve reduction in the skin colonisation on day seven though it claimed an extended antiseptic effect until 72 hours differentially in various sites.

Our study was able to achieve a sustained reduction in skin colonisation during the first week. It was the first one to demonstrate the impact of chlorhexidine after multiple applications. The biological rationale for this was the substantivity of chlorhexidine which would last 48 hours⁴⁰. A single application at birth may not produce a sustained effect until one week. This fact is also substantiated by the conclusion from the latest systematic review (2013) on this topic.⁵⁵

Multiple cleansings with chlorhexidine was able to achieve greater reduction in colonisation (50%) than a single application (33%). There seems to be a cumulative effect in reduction of colonisation with multiple applications. This reduction in colonisation during the first week may offer long-standing protection against the transcutaneous route of entry.

A significant reduction in the colonisation rates could not be achieved at 24 hours after a single application both in the axilla and groin regions. However a single application of 0.5% chlorhexidine was able to reduce skin colonisation by 33% and 28% in the axilla and groin respectively at 48 hours. Our study demonstrated a quantitative reduction in colony counts also in the culture positive swabs at 48 hours and on the seventh day 7 in the axilla and groin which was statistically significant.

In a hospital based study in Bangladesh ⁵² at 24 hours colonisation was significantly less with chlorhexidine in both axilla and groin. The study included larger, mature neonates (mean B.W= 3000gms) who would possess better skin maturity and are at less risk of nosocomial skin colonisation. They excluded infants weighing less than 1500 g who are predisposed to nosocomial colonisation and sepsis due to their longer hospital stay.

The study⁴⁸ in Nepal among hospital born newborns demonstrated 48% and 50% reduction in the risk of colonisation with 0.25% and 0.5% chlorhexidine respectively at 2 hours after single application. Since more than 50% of neonates were discharged earlier it could not report significant reduction at 24 hours. There was a dose response trend on skin colonisation. The highest

concentration (1%) achieved 63% reduction followed by 0.5% achieving 50% at 2 hours.

But both the concentrations could not achieve reduction at 24 hours, a period when the skin colonisation in neonates increase exponentially after birth. The study at AIIMS among the preterm population⁵³ reported 60% reduction in the axillary colonisation at 24 hrs after using 0.25% chlorhexidine. There was no significant reduction in the groin.

Thus the results have been variable and inconclusive in the literature. We understand from the previous trials that 0.25% concentration of chlorhexidine did not achieve a reduction in skin colonisation consistently after a few hours.

We were able to achieve significant difference in the colonisation after a single application consistently both in the axilla and groin even at 48 hours. Using chlorhexidine at higher concentration (0.5%) achieved additional efficacy and greater residual activity. A 50% reduction in the risk of colonisation by 0.5% chlorhexidine in the study conducted by Luke C Mullany et al in Nepal⁴⁸ was similar to our result. The increased efficacy of higher concentrations of chlorhexidine is explained rationally by its bactericidal action at higher concentrations⁴⁰.

In our study the difference in skin colonisation between the placebo and intervention groups was not significant at 24 hours. We speculate that the natural exponential increase^(3,33) in skin colonisation rates after 12- 24 hours in both the study groups would not have allowed to achieve significant difference between them.

This is evidenced by the increase seen in colonisation rates in the placebo group above the baseline at 24 hours compared to the chlorhexidine group. Also some of the previous studies were not able to show reduction in the rates at 24hrs after the reduction achieved at 2 hours due to the rebound increase in colonization^(48,54). At 48hours the colonisation in the placebo group increased unintervened as compared to the decreased colonisation in the intervention group. Hence at 48 hours there was a statistically significant difference between them.

The axillary colonisation rate was initially higher than the groin at baseline. Subsequently colonisation rates increased in the groin. A logical explanation would be that the axilla would be handled more frequently at admission in the process of transportation from the labour ward to the NICU, during weighing and various procedures of care and treatment. Afterwards groin area gets contaminated more by flora from nearby perineum and hands of the caregivers during the process of changing diapers. The higher concentration of chlorhexidine used in our study produced consistent results in both the areas studied. This concentration was effective even in the inguinal region.

We did not study the peri umbilical colonisation .Few studies included periumbilical region also^(48,52). Many times skin probes are attached to this region with adhesives and may interfere with the results and hence was not a choice in our study.

Though the babies are considered to be sterile at birth and colonisation only gradually increases from 6-12 hrs of postnatal life ^(3,33), the baseline skin

colonisation in the control group in the axillary and groin regions were 44.3% and 37.5% respectively. This is similar to the baseline line smears reported in the AIIMS trial (30% & 40%). The baseline growth in the intervention group is higher than the placebo group at both the axillary (64.4%) and inguinal (62.5%) sites.

The studies conducted by Luke C Mullany et al⁴⁸ in inborn neonates and Gary L Darmstadt et al⁵² in out born neonates admitted to the unit within 72 hrs have reported similar rates of colonisation at the baseline.

The baseline colonisation in the chlorhexidine group was significantly higher than the placebo groups unlike the other studies. This could be due to the unequal distribution of the mothers of different socioeconomic strata in the two study groups. The higher proportion of mothers of lower socio economic class were in the chlorhexidine group as compared to the placebo group. The poor hygienic status of the mothers in the lower socioeconomic class could have predisposed to the higher baseline skin colonisation in the chlorhexidine group.

Mortality rates and incidence of sepsis

The Neonatal mortality rate and the incidence of sepsis were not different in both the study groups. Before and after studies done in Egypt⁵⁴ and Malawi¹³ hospitals and the communities in Nepal¹² were able to show a reduction in neonatal mortality with the chlorhexidine intervention. But later studies which were randomised controlled trials like ours were not able to show a reduction.

A systematic review (2013)⁵⁵ of these studies concluded that a single application may not produce an effect on the mortality rates and incidence of

sepsis. Repeat hospitalisation rates and the proportion remaining sick on day -28 of life were significantly lower in the experimental group. The reduction in skin colonisation achieved with multiple applications of chlorhexidine in our study did not get translated as reduction in mortality and sepsis rates in the infants. A study with a larger sample size may produce a difference.

However there was a reduction in the incidence of sepsis in the more immature infants between of 28 - 31 weeks of gestation GA in the subgroup analysis. This is clinically relevant and important because in the smaller preterm infants the transcutaneous route of entry is likely to be more common than the mature ones and chances of sepsis caused by resident micro flora of the skin like CoNS is more prevalent in this group¹³. Thus these immature preterm infants with most handicaps in their defense mechanisms are likely to be benefitted by the antiseptic action of chlorhexidine.

Safety profile of chlorhexidine

Our study showed that the concentration of chlorhexidine at levels of 0.5% was well tolerated by the preterm neonates. The reports from our study demonstrate the safety profile of chlorhexidine in preterm neonates even after multiple applications in terms of temperature stability and skin integrity.

The incidence of cold stress and hypothermia was not significant in our study population. The latest systematic review (2013) quoted on this topic that hypothermia was a significant concern with chlorhexidine application in the preterm infants.⁵⁵ Our babies were cleansed most of the times under radiant warmers and rarely in the postnatal wards. Previous studies by Darmstadt and

Sankar ^{52, 53} done under similar conditions have also not reported significant hypothermia.

There were no signs of erythema, fissuring or dryness in the skin. Skin condition scores even after repeated cleansing were similar to the baseline scores in both the study groups. The chlorhexidine was used in the aqueous form avoiding the alcohol which could have been the culprit for the skin burns and erythema as previously reported in the literature⁴³. There were reports of chlorhexidine causing dermatitis only when it was impregnated in the dressings placed over catheter sites restricting the capillary perfusion at that site⁵⁷. Few ELBW infants developed erythema and 3 or 4 infants had skin break down and exudation with a high concentration of chlorhexidine in the study by Bringue et al ⁴⁶ (2%). There were no reports of contact dermatitis when chlorhexidine was used for whole body cleansing, in aqueous form and in 0.5% concentration even in the earlier studies. ^(48,52,53)

Skin colonisation profile

It was feared initially that multiple applications of chlorhexidine would ironically increase the sepsis rates because reduction in the resident flora might predispose to colonisation by pathogenic, nosocomial gram negative organisms. This effect could be compounded much more due to the fact that chlorhexidine was chosen to be applied in the aqueous form without alcohol, losing the cidal effect of alcohol on the gram negative organisms⁴⁵. It was found not only to be safe but also did not increase the risk of nosocomial gram negative colonisation .

Systemic absorption of chlorhexidine

The systemic absorption of chlorhexidine was not assayed in our study. Systemic absorption in preterm infants exposed to chlorhexidine would be a significant area of concern because the thin epidermal layer is ineffective in protecting against the absorption of toxic substances applied topically. There are no established values for what is considered to be a safe concentration of chlorhexidine in the blood⁵⁷.

However we limited our applications to three in the study since the much feared neurotoxicity in the hexachlorophene usage through systemic absorption from the skin was reported after four or more applications⁴⁷. The alcohol that is known to potentiate the absorption of topically applied chlorhexidine⁴⁹ was also avoided in our study. We skipped the areas like face and scalp which are highly vascular³ and would increase the chances of systemic absorption. Even then to document its safety with a full proof a long term neuro developmental follow up of the infants may be necessary. None of the studies in the literature also have attempted to study this aspect of its safety profile⁵⁷.

Strengths of the study

Our study came up with a new idea of multiple applications of chlorhexidine based on its property of substantivity for the first time in neonatal medicine.

Our study is the first RCT with a large sample size in preterm population.

The double blinding was full proof eliminating treatment and measurement bias.

The higher concentration of chlorhexidine demonstrated the sustained reduction in skin colonisation for the entire first week consistently in both the areas studied.

Limitations of the study

Failure to assay the serum levels of chlorhexidine after multiple applications would be an important limitation of our study.

The study did not report on the neurodevelopmental outcome of the infants to detect any potential neurotoxicity.

CONCLUSION

CONCLUSIONS

- 1) Multiple applications of 0.5% chlorhexidine reduced the risk of skin colonisation in the axilla and groin by 50% and 38% respectively during the first week (NNT = 2).
- 2) A single application of 0.5% chlorhexidine at 48 hours reduces the risk of skin colonisation by 33% and 28% in the axilla and groin respectively.
- 3) The results of quantitative reductions in the bacterial colony counts in the axilla and the groin at different time points were also similar.
- 4) There was no difference in mortality on the 28 – day of life between the two groups.
- 5) There was no significant difference in the incidence of sepsis during the first week of life between the groups. But the incidence of sepsis in the 28-31 weeks GA infants was lower in the chlorhexidine group.
- 6) Rate of repeat hospitalisation and the proportion remaining sick on day – 28 of life were significantly lower in the intervention group.
- 7) Multiple applications of 0.5% chlorhexidine in the aqueous form was well tolerated by the preterm infants without skin toxicity and thermal instability. The aqueous preparation did not predispose to skin colonisation with gram negative organisms.

Implications for practice

Preterm neonates admitted in the NICUs may benefit from skin cleansing with 0.5% Chlorhexidine wipes every alternate day during the first week to reduce skin colonisation.

Implications for research

- 1) The neurodevelopmental outcome of the preterm neonates cleansed with chlorhexidine in the nurseries should be studied on a long term follow up.
- 2) The synergistic effect of combining neonatal intervention with maternal vaginal cleansing during delivery can be studied.

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ANNEXURES

Annexure -1A

INSTITUTIONAL ETHICS COMMITTEE

MADRAS MEDICAL COLLEGE, CHENNAI - 3

EC Reg No. ECR/270/Inst./TN/2013
Telephone No.: 044 25305301
Fax : 044 25363970

Certificate of approval

To

Dr. M. Anitha,
D. M (Neonatology) post graduate,
Institute of child health and Hospital for children, Egmore,
Madras Medical college , Chennai-3

Dear Dr. M. Anitha,

The Institutional Ethics Committee of Madras Medical college, reviewed and discussed your application for approval of the proposal entitled " *Efficacy of whole body cleansing with multiple +applications of chlorhexidine in haemodynamically stable 28-34 weeks of completed gestational age neonates admitted in a tertiary care hospital – A double blinded randomized trial*". No. 25112013.

The following members of Ethics Committee were present in the meeting held on 13.11.2013 conducted at Madras Medical college, Chennai-3.

- | | |
|--|-----------------------|
| 1. Dr. G. Sivakumar, MS, FICS, FATS |Chairman |
| 2. Prof. R. Nandini, MD |member secretary |
| Director Instt. of Pharmacology, MMC, Ch-3 | |
| 3. Prof. Ramadevi |member |
| Director i/c Instt. of Biochemistry, ch-3 | |
| 4. Prof. P. Karkuzhall, MD |member |
| Prof. Instt. of Pathology, MMC, Ch-3 | |
| 5. Prof. Kalaiselvi, MD |member |
| Prof. of Pharmacology, MMC, Ch-3 | |
| 6. Thiru. Govindasamy, BABL |Lawyer |
| 7. Tmt. Arnold saulina, MA MSW |social scientist |

We approve the proposal to be conducted in its presented form.

Sd/ Chairman & other members

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients' information / informed consent and asks to be provided with a copy of the final report.

R Nandini 25/11/13
Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE

Annexure 1-B



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Introduction

Preterm birth is truly a global problem. Countries with highest numbers include Brazil, India, Nigeria and the United States of America.¹ In the poorest countries on an average, 12% of babies are born too soon compared with 4% in higher income countries.² Preterm infants due to their immaturity of various organ systems are more likely to be admitted to the neonatal intensive care units than their term counterparts who are likely to be by their mother side. During the process of birth, transport to NICU and various procedures of treatment and care, infant's skin gets colonized by flora derived from the body of the mother, other human contacts and various inanimate objects.³

The mechanisms leading to colonization of the skin involve a complex interplay among rapid growth of commensal organisms, the development of the acid mantle, local immune environmental factors such as occlusion and humidity and the choice of exogenous soaps and skin care practices.⁴ Initial colonization is primarily depends on the first viable organism to arrive at a particular site as well as factors such as type of delivery, the amount of contact present at birth, the type of nourishment received and the degree of exposure to the hospital environment.⁵ Though microbial colonization begins immediately after birth, it is low at onset and the rate increases after 12 hours.⁶

Annexure 1-C

4/10/2014

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Annexure - 2

Data collection and entry form

Name:

Serial no:

Study random no:

Infant Details

IP no:

Disc no:

Address:

Date / time of admission

Date / time of Birth

Date / time of recruitment

Contact Phone no.

Gestational age by dates :

USG:

NBS

Birth weight: gms (AGA /SGA /LGA)

Sex:

Mode of delivery:

Resuscitation details: Routine/ initial steps/ O2 /BMV/ intubation / chest comp

Post resuscitation: Respiratory = O2 / CPAP/ mech ventilation

Circulatory = Inotropes 1/ Ionotrope 2 / Adrenaline

Encephalopathy = Yes / no

Skin defects:

Vernix:

Major congenital malformations:

Sepsis Score:

Empirical antibiotics: Yes / No

Immediate referrals :

Maternal details

Serial no:

Name of the mother & Father

Monthly income:

IP no:

Educational status of father:

Age:

Educational status of Mother:

ANC : visits:

Occupation:

Date of admission:

Socio economic Status

Date of delivery:

LMP:

EDD:

Gestational age by dates:

Gestational age by USG

Gestational illness: GDM/ PIH /Anemia / others

Drug intake:

Reason for Prematurity:

Labour induction:

ANCS details:

Risk factors for EOS

- i. Maternal fever
- ii. lower abdominal pain / Dysuria
- iii. PROM
- iv. Foul smelling liquor

- v. No of PV
- vi. Fetal tachycardia
- vii. Duration of labour
- viii. e/o fetal distress, Meconium stained liquor
- ix. Intrapartum Antibiotics

Placenta:

NEWBORN SKIN CONDITION SCORES

	At birth	Before 2 nd wipe	Before 3 rd wipe	End of study
Date				
Time				
Score				

AXILLARY TEMPERATURE

After wash	Date	Time	0 min	5 min	15 min
1 st					
2 nd					
3 rd					

Smear	Date	Time	Axilla		Inguinal	
			organism	Colony counts	organism	Colony counts
Baseline						
24 hrs						
48 hrs						
D7						

SNo	DATE:	D1	D2	D3	D4	D5	D6	D7	Comments
	DAY:								
1.	RESPIRATORY								
2.	CIRCULATORY								
3.	SENSORIUM								
4.	SEPSIS SYMPTOMS								
	SIGNS								
5.	FOCUS								
6.	SKIN SCORE								
7.	FEEDING								
8.	IVF								
9.	ANTIBIOTICS								
10.	UMBILICUS								
11.	IV LINE								
12.	CENTRAL								

14.	INVASIVE								
	PROCEDURE								
15.	Xray								
16.	TLC								
	pH								
17.	ANC								
18.	CRP								
19.	Micro ESR								
20.	Blood C/S								
21.	CSP Analy								
22.	CSP 9S								
23.	Other 9S								
24.	Mix Culture								

SYMPTOMS & SIGNS OF SEPSIS with day of onset

- | | | | |
|----|---------|------------|---------|
| 1. | () | 6. | () |
| 2. | () | 7. | () |
| 3. | () | 8. | () |
| 4. | () | 9. | () |
| 5. | () | 10. Others | () |

Discharge / death

Date & Time =

Day of life

FOLLOW UP

1.Repeat hospital admissions

2.Status on Day 28 of life

Date :

ANNEXURE – 3A

ஆராய்ச்சி ஒப்புதல் படிவம்

பச்சிளம் குழந்தைகளின் சருமத்தில் உள்ள நுண்கிருமிகளை
அகற்றுவதில் குளோர்ஹெக்ஸிடின் உடைய ஆற்றலை
கண்டறியும் ஆய்வு.

ஆராய்ச்சியாளரின் பெயர்:

வரிசை எண்.

குழந்தையின் பெயர்:

தேதி:

.....என்னும் ஆண்/ பெண் (அடையாள எண்.....)
குழந்தையின் பெற்றோர், திரு.....ஆகிய நான்,

என் குழந்தை குறைமாதகமாகவும், எடைகுறைவாக உள்ளதையும்
மருத்துவர்களிடமிருந்து அறிந்துகொண்டேன். அதனால் குழந்தையின் நோய்
எதிர்ப்பு சக்தி குறைந்து கிருமிகளால் தாக்குண்டு உயிரிழக்கும் அபாயம்
உள்ளது என்பதையும் அறிந்துகொண்டேன். எனவே நான் குளோர்ஹெக்ஸிடின்
பயன்படுத்தி மேற்கொள்ளப்படும் இந்த ஆராய்ச்சியில் பங்குகொள்ள ஒப்புதல்
அளிக்கிறேன். அதன் மூலம் என் குழந்தையின் உடலை குளோர்ஹெக்ஸிடின்
அல்லது தூய்மையான நீரை கொண்டு துடைக்க சம்மதிக்கிறேன். இந்த
ஆராய்ச்சியில் என் குழந்தைக்கு தேவையான மருத்துவ பரிசோதனைகளை
செய்யவும் ஒப்புதல் அளிக்கிறேன்.

மருத்துவர்கள் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் தன்மையைபற்றி
எனக்கு விளக்கியுள்ளனர். அதை முழுமையாக புரிந்துகொண்ட நிலையில்
இந்த ஒப்புதலை நான் அளிக்கிறேன். இந்த ஆராய்ச்சியில் பங்குகொள்வதினால்
என் குழந்தையின் உடல்நிலைக்கு தேவைப்படும் மருத்துவ சிகிச்சை
எவ்விதத்திலும் மாறாது என்பதை அறிவேன். என் முழுமையான சுயநினைவுடன்
இந்த ஒப்புதலை நான் அளிக்கிறேன். இந்த ஆராய்ச்சியில் பங்குகொள்ள
மறுத்தாலும், என் குழந்தைக்கு தேவையான மருத்துவ சிகிச்சைகள் தடையின்றி
நடைபெறும் என்பதை அறிவேன். இந்த ஆராய்ச்சியில் பங்குகொள்ள
எந்தவிதமான வற்புறுத்தலும் இல்லை என்றும், நான் விரும்பினால் ஆராய்ச்சியின்
எந்த கட்டத்திலும் என் குழந்தையை விலக்கிக்கொள்ளலாம் என்பதையும்
அறிவேன்.

ஆராய்ச்சியாளர் கையொப்பம்

பெற்றோர்/ காப்பாளர் கையொப்பம்

ANNEXURE – 3B

ஆராய்ச்சி தகவல் தாள்

பச்சிளம் குழந்தைகளின் சருமத்தில் உள்ள நுண்கிருமிகளை
அகற்றுவதில் குளோர்ஹெக்ஸிடின் உடைய ஆற்றலை
கண்டறியும் ஆய்வு.

ஆராய்ச்சியாளரின் பெயர்:

வரிசை எண்.

குழந்தையின் பெயர்:

தேதி:

எடை குறைவாக பிறக்கும் குறைமாத பச்சிளம் குழந்தைகளுக்கு பொதுவாக நோய் எதிர்ப்பு சக்தி மிககுறைவாக இருக்கும். அதனால் அவர்கள் எந்நேரத்திலும் கிருமிகளால் தாக்குண்டு உயிர் இழக்கும் அபாயம் உள்ளது. எனவே, அவர்களின் சருத்தின் மேலுள்ள கிருமிகளை கிருமிநாசினி கொண்டு அகற்றுவதன்மூலம் இந்த குழந்தைகளுக்கு அதிக பாதுகாப்பு கொடுக்க முடியும் என்று நம்பப்படுகிறது. குளோர்ஹெக்ஸிடின் என்னும் கிருமிநாசினியை நாம் குழந்தைகளின் சருமத்தில் துடைக்க பயன்படுத்தும்பொழுது அது கிருமிகளை கொன்று, அவற்றினால் நோயுண்டு உயிரிழக்கும் அபாயத்திலிருந்து அக்குழந்தைகளை காக்க முடியும் என்று விஞ்ஞான ஆராய்ச்சிகள் தெரிவிக்கின்றன. ஆனால் இந்த மருந்தினை உபயோகிக்கும் அளவு, பயன்படுத்தும் முறை, மருத்துவ பயன்பாடுகள், பின்விளைவுகள் ஆகியவை துள்ளியமாக கண்டறியப்படவில்லை எனவே, இந்த மருத்துவ மனையில் பிறக்கும் எடை குறைந்த குறைமாத பச்சிளம் குழந்தைகளின் சருமத்தில் இம்மருந்தினை உபயோகித்து மேற்சொன்னவற்றை ஆராய்ச்சியின்மூலம் விஞ்ஞான பூர்வமாக கண்டறிய உள்ளோம். எங்கள் ஆராய்ச்சியின் விதிமுறைகளுக்கு உட்பட்டதாக உங்கள் குழந்தைகள் தேர்வு செய்யப்படுமேயானால் பெற்றோர்களாகிய உங்களின் ஒப்புதலுடன், உங்கள் குழந்தை ஆராய்ச்சியில் பங்குபெறும் வாய்ப்பினை பெறும் இந்த ஆராய்ச்சியின் கீழ் உள்ள இரண்டு குழுக்களில் ஒன்றில் உங்கள் குழந்தை சேர்க்கப்படும்.

இரண்டு குழுவில் உள்ள குழந்தைகளும் சருமத்தை தூய்மை செய்யும் மிருதுவான பஞ்சினால் முதல் வாரத்தில் மூன்று முறை துடைக்கப்படுவார்கள் அதன்பிறகு அவர்களின் சருமத்தில் உள்ள கிருமிகளை ஆராய் மருத்துவ சோதனைகள் மேற்கொள்ளப்படும். ஒரு குழுவில் குழந்தைகள்

குளோர்ஹேக்ஸிடின் மருந்தினால் துடைக்கப்படுவார்கள் மற்றொன்றில் தூய்மையான நீரினால் துடைக்கப்படுவார்கள் எந்த குழந்தை எந்த மருந்தினால் துடைக்கப்படுகிறது என்ற விவரம் சிகிச்சை அளிக்கும் மருத்துவர்களுக்கோ, செவிலியர்களுக்கோ மருத்துவ சோதனைகளை செய்பவர்களுக்கோ, ஆராய்ச்சி மேற்கொள்பவர்களுக்கோ தெரிவிக்கப்படாமாட்டாது. அதன் விவரங்கள் ஆராய்ச்சியின் முடிவிலோ அல்லது உயிர்க்கு ஆபத்து ஏற்படும் நிலையிலோ மட்டும் வெளியிடப்படும்.

முன்னால் மேற்கொள்ளப்பட்ட ஆராய்ச்சிகளில் இந்த மருந்தினால் சருமம் ஜில்லிட்டு போகும் என்றும், சருமத்தில் சிவந்த படைகள் ஏற்படலாம் என்றும் கண்டுபிடிக்கப்பட்டுள்ளது. அதனை தவிர்க்கும்பொருட்டு குழந்தைகள் தக்க வெப்பமூட்டும் முறைகளுடன் ஆராய்ச்சியில் ஈடுபடுத்தப்படுவார்கள். மேலும் அவர்களின் சரும நிலை குறிப்பிட்ட கால இடைவேளைகளில் கண்காணிக்கப்படும். அப்பொழுது சருமநிலை வெகுவாக பாதிக்கப்பட்டு வெடிப்புகள் ஏற்படுமானால், அதன்பிறகு மருந்துகள் துடைப்பதற்கு உபயோகப்படுத்தப்படமாட்டாது. சருமநிலை குணமடைய தக்க சிகிச்சை அளிக்கப்படும்.

இந்த ஆராய்ச்சியில் உங்களின் பங்கேற்பு எந்தவிதத்திலும் உங்கள் குழந்தையின் உடல்நிலைக்கு தேவைப்படும் சிகிச்சையை மாற்றுவதாகவோ, தடைசெய்வதாகவோ இருக்காது. குழந்தைகள் ஒருமாதம் வரை அவர்கள் தொடர்காணலுக்காகவும், சிகிச்சைக்காகவும். தடுப்பூசி போடும் பொழுதும் இம்மருந்துவமனைக்கு வரும்பொழுது கண்காணிக்கப்படுவார்கள்.

இந்த ஆராய்ச்சியில் உங்கள் பங்கேற்பு மிக ரகசியமாக வைக்கப்படும். ஆராய்ச்சியின் முடிவுகளை தங்கள் விருப்பத்தின்பேரில் தெரிந்துகொள்ளலாம். ஆராய்ச்சியின் முடிவுகளில் உங்களின் தனிப்பட்ட அடையாளம் ஏதும் இருக்காது. இந்த ஆராய்ச்சியில் பங்குகொள்ள நீங்கள் சுதந்திரமாக முடிவு எடுக்கலாம். ஆராய்ச்சியின் எந்த கட்டத்திலும் நீங்கள் விரும்பினால் விலகிக்கொள்ளலாம். நீங்கள் பங்குகொள்ள மறுத்தாலும், உங்கள் குழந்தைக்கு தேவையான மருத்துவ சிகிச்சைகள் அனைத்தும் தடையின்றி வழக்கம்போல் மேற்கொள்ளப்படும்.

ஆராய்ச்சியாளர் கையொப்பம்

பெற்றோர்/ காப்பாளர் கையொப்பம்

ANNEXURE – 4 A

CONSENT FORM

Effect of Whole body cleansing with chlorhexidine on skin colonization in newborns. – a randomised trial

Name of investigator:

Date:

Name of the patient :

Serial number:

I Ms/Mr. _____ M/O//F/O, B/O _____
Sex _____ Hosp. No. _____ admitted in the Neonatal ICU of IOG , Egmore was explained by the doctor that my baby is born preterm and low birth and therefore more vulnerable to infections from microorganisms on the skin surface . I am willing to participate in this trial and I give my consent to apply chlorhexidine or sterile water on the skin using baby wipes as per the study protocol.

I also give my consent for taking swabs from the skin needed during the study. The adverse effect like skin rashes, cold stress were explained to me.

The doctors have explained to me the nature and the purpose of the trial. I have given my consent only after completely understanding the details that were explained to me. I understand that my baby's routine clinical management is not affected by my participation in this trial.

I have given this consent to be enrolled in this study with my full consciousness. I am willing for my baby to be enrolled in this study without any ones compulsion and I am fully aware that I can withdraw from the trial at any time during the study.

Signature of Investigator

Signature of Parent

Annexure – 4B

INFORMATION SHEET

Effect of Whole body cleansing with chlorhexidine on skin colonization in newborns. – a randomised trial

Name of investigator:

Date:

Name of the patient :

Serial number:

Many microorganisms are present on the body surface of the newborn infants. The preterm Low birth weight infants have weak skin barrier and defense mechanisms. Hence these organisms go into blood and cause serious infections and death. Chemicals like chlorhexidine can reduce or remove these organisms from the skin surface and protect these infants. But details regarding appropriate dose, safety and clinical benefits are unknown in preterm infants. Hence we are conducting a scientific experiment by using chlorhexidine to decrease the surface microorganisms in preterm infants admitted in our newborn unit at Institute of Obstetrics & Gynaecology.

After obtaining your consent, your baby will be included as a participant in the trial if he/she satisfies our eligibility criteria. The participants will be included under one of the two study groups. The participants will be wiped with body wipes applying the content over the entire skin surface except on the face every alternate day during the first week of life (3 times). The content will be chlorhexidine in one study group and sterile water in the other study group. Swabs will be taken from the skin surface to detect and count the organisms before and after the application. The contents of the wipes shall not be disclosed to the caregivers and the professionals involved in the treatment and lab investigations. The treatment code will be broken after the analysis or in the event of life threatening adverse effects.

As known from the previous experiments, during the procedure the baby may experience cold stress. The baby will be nursed under a radiant warmer, body temperature will be monitored at regular intervals and necessary warmth will be given appropriately. After application the skin may appear red, dry or breakdown. Further application will be stopped and appropriate treatment will be given in case of serious skin breakdown.

Your participation in the trial will not influence the routine clinical management necessary for the condition of the participant. The participants will be under follow up after discharge for one month of life during their visits to hospitals for medical checkups and immunizations or through telephonic calls.

The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared. Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which your child is otherwise entitled. The results of the study may be intimated to you at the end of the study period.

Signature of investigator

Signature of Parent

Annexure -5

Socioeconomic Status Scale of Kuppusamy

(Urban 1976), Score card

(A) Education score

1. Professional or honours	7
2. Graduate or post-graduate	6
3. Intermediate or post high school diploma	5
4. High school certificate	4
5. Middle school certificate	3
6. Primary school or literate	2
7. Illiterate	1

(B) Occupation

1. Profession	10
2. Semi- profession	6
3. Clerical, shop owner, farmer	5
4. Skilled worker	4
5. Semi-skilled worker	3
6. Unskilled worker	2
7. Unemployed	1

(c) Family income per month(in Rs) (Modified for 1998)

)	Original	Modified	
1.	>2000	>13500	12
2.	1000-1999	6750-13499	10
3.	750-999	5050-6749	6
4.	500-749	3375-5079	4
5.	300-499	2025-3374	3
6.	101-299	676-2024	2
7.	<100	<675	1

Total score	Socioeconomic class	
1. 26-28	upper (I)	
2. 16-25	Middle	Upper middle(II)
3. 11-15	Lower middle(III)	
4. 5-10	Lower	Upper lower (IV)
5. <5		Lower (V)

Annexure - 6

Newborn skin condition scoring scales

Dryness:

- 1 = Normal , no sign of dryness
- 2 = Dry skin , visible scaling
- 3 = very dry skin,cracking/fissures

Erythema:

- 1 = No evidence erythema
- 2 = Visible erythema, <50% body surface
- 3 = Visible erythema, >50% body surface

Breakdown:

- 1 = None evident
- 2 = Small, localised areas
- 3 = Extensive

Perfect score = 3

Worst score = 9

S. No.	Strat cd	Randc	Group	Maternal Name	Recru age	GA	days	B.W	Sex	Mult
1	B	1	CHX	srisha	180	34	1	1810	2	
2	B	2	CHX	kamatchi	120	34	2	2150	1	
3	B	3	PLB	Vannila	135	34	1	2640	1	
4	A	1	CHX	Vasanthi	235	31	4	1690	1	
5	B	4	PLB	chandra	105	34	2	1700	2	
6	A	2	PLB	anitha	60	31	5	1770	1	
7	A	3	CHX	Jothi	360	31	0	1150	2	
8	B	5	PLB	chinna nagamma	90	34	3	2095	1	
9	B	6	CHX	Kavitha	240	34	6	1895	2	
10	B	7	CHX	Grace	300	32	0	1505	2	
11	A	4	PLB	Madina	180	31	5	1745	1	
12	B	8	CHX	Srimathy	240	32	6	1590	1	
13	B	9	PLB	Ishwarya	15	33	6	1100	1	
14	A	5	CHX	Jayalakshmi	360	29	2	1050	2	
15	B	10	PLB	Saraswathy	180	32	3	2050	1	
16	B	11	PLB	Kavitha 3	120	34	4	1895	1	TCTA
17	B	12	CHX	Kavitha 2	120	34	4	1400	1	TCTA
18	B	13	CHX	Rajeswari	60	34	0	1360	2	
19	B	14	PLB	Asma siddika	60	32	0	1820	2	
20	B	15	PLB	Asha	210	34	3	1870	1	
21	B	16	CHX	Ratna	90	33	0	1855	2	
22	B	17	PLB	Vasanthi	300	34	5	2335	2	
23	B	18	CHX	Patchaiammal	150	34	0	2080	1	
24	B	19	CHX	Amulu	60	33	4	1125	1	
25	A	6	CHX	Shenbagavalli	330	31	6	1120	1	
26	B	20	CHX	Vanaja	240	34	2	1900	1	
27	B	21	PLB	Suguna	60	34	6	2500	2	
28	B	22	PLB	Malarkodi	180	34	5	1855	2	
29	A	7	PLB	Kokila	120	31	6	1140	2	
30	B	23	PLB	Divya Bharathi	60	32	5	1450	1	
31	B	24	PLB	Indumathy	300	34	4	2510	1	
32	A	8	PLB	Sofiamary	60	31	6	1230	1	
33	B	25	CHX	Jeyashree	120	32	6	1890	2	
34	B	26	CHX	Prasanna 1	180	33	4	2295	2	MCMA
35	B	27	CHX	Prasanna 2	180	33	4	2160	2	MCMA
36	B	28	PLB	Puspalatha 1	300	34	2	2225	2	MCDA
37	B	29	PLB	Puspalatha 2	300	34	2	2240	2	MCDA
38	B	30	CHX	Kamala	240	32	4	1820	2	
39	B	31	CHX	Nithyamalli	360	33	6	1920	2	
40	B	32	PLB	G0wsiya begam	210	34	5	2025	2	
41	B	33	CHX	Sudha	120	32	2	1660	1	
42	A	9	CHX	Subbalakshmi 1	120	29	2	1315	2	MCDA
43	A	10	PLB	Subbalakshmi 2	120	29	2	1270	2	MCDA
44	B	34	CHX	Muzhumathi	60	34	3	2990	2	
45	B	35	PLB	Nithya	300	34	0	2075	1	
46	B	36	PLB	Usha 1	60	33	4	1580	2	MCDA
47	B	37	PLB	Usha 2	60	33	4	1665	2	MCDA
48	A	11	PLB	Kavitha	300	31	2	1135	1	
49	A	12	CHX	Janaki	180	28	4	1100	1	

50	B	38	PLB	Usha	180	32	5	1815	2	
51	B	39	CHX	Vanaja	90	34	0	1940	2	
52	B	40	CHX	Sivasakthi	330	34	5	2000	2	
53	B	41	PLB	Bharathi 2	180	34	2	1300	2	DCDA
54	B	42	CHX	Bharathi1	180	34	2	2045	2	DCDA
55	B	43	PLB	Malathy	120	34	0	1900	1	
56	A	13	CHX	Bama	120	31	6	1165	2	
57	B	44	CHX	Asha 1	260	33	5	1660	2	MCMA
58	B	45	CHX	Asha 2	260	33	5	1420	2	MCMA
59	A	14	PLB	Chithra 2	360	31	5	1070	2	DCDA
60	A	15	CHX	chithra 1	360	31	5	1550	1	DCDA
61	B	46	PLB	Nagendra mani	105	33	0	1660	1	
62	A	16	PLB	Dhanalakshmi	120	30	5	1185	2	
63	B	47	CHX	Poongavanam 1	300	33	5	1380	2	MCDA
64	B	48	CHX	Poongavanam2	300	33	5	1245	2	MCDA
65	B	49	PLB	shruti	240	33	5	1900	2	
66	A	17	CHX	Sheerin 1	60	30	1	1430	1	MCDA
67	A	18	PLB	Sheerin 2	60	30	1	1345	1	MCDA
68	B	50	PLB	Ambika	80	34	0	2090	1	
69	A	19	CHX	Jayanthi	90	31	1	1655	1	
70	A	20	PLB	Ammu	120	30	1	1330	2	
71	B	51	CHX	Indra	240	34	4	1715	1	
72	A	21	PLB	Bharathi 2	315	29	5	1300	1	MCDA
73	A	22	CHX	Bharathi 1	315	29	5	1740	1	MCDA
74	B	52	CHX	Boomadevi	360	33	3	2300	1	
75	B	53	PLB	Easwari	295	33	2	2510	1	
76	B	54	PLB	Thulasi	180	32	7	1500	1	
77	B	55	PLB	Parimala	330	34	5	1930	2	
78	B	56	PLB	Sumathy	150	34	4	2365	2	
79	A	23	CHX	Karthiga selvi	180	28	2	1190	1	
80	B	57	CHX	Karishma	120	34	2	1955	1	
81	B	58	CHX	Kaliyammal	40	33	4	1050	2	
82	A	24	CHX	lakshmi	300	30	2	1365	1	
83	B	59	PLB	Chandrammal	240	33	0	2115	2	
84	B	60	CHX	Saranya	270	33	0	1685	1	
85	B	61	PLB	Indumathy 2	75	34	4	2040	2	DCDA
86	B	62	CHX	Indumathy1	75	34	4	2360	2	DCDA
87	A	25	PLB	Geetha	60	30	2	1370	1	
88	B	63	PLB	Kalpana	270	34	1	2005	2	
89	B	64	CHX	Dhanalakshmi	150	34	4	1935	1	
90	B	65	CHX	Jayanthi	300	34	5	2275	2	
91	B	66	CHX	Venda	70	33	5	1340	1	
92	B	67	PLB	Mouli	90	32	6	1590	1	
93	B	68	PLB	Jansirani	200	32	6	1755	2	
94	A	26	PLB	Yasmin	240	30	3	1473	1	
95	B	69	PLB	Hemalatha	180	33	1	1790	1	
96	A	27	CHX	Karpagam 1	300	31	1	1310	1	MCMA
97	A	28	PLB	Karpagam 2	300	31	1	1500	1	MCMA
98	B	70	CHX	Ammu 1	270	34	5	1800	2	MCTA
99	B	71	CHX	Ammu 2	270	34	5	1260	2	MCTA

[illegible]

Vernix	Matern	Social s	status c	mater	ANC visit	mode	Admin-	adm del	ANCS	Preterm d	pPROM
1	22	12	2	2	4	1	9	1	1	1	2
1	27	7	1	1	3	1	12	1	1	1	2
1	22	24	3	3	4	4	7	1	3	2	1
1	27	13	2	2	3	1	11	1	1	1	2
1	21	11	2	2	4	1	4	1	1	1	2
2	19	16	3	2	2	1	10	1	3	3	2
1	20	17	3	2	5	4	8	1	3	1	2
2	23	14	2	1	1	1	6	1	1	1	2
2	21	13	2	1	4	2	96	4	1	3	2
2	26	25	3	2	3	4	6	1	3	3	2
1	21	23	3	2	3	4	144	4	3	1	2
1	26	19	3	1	4	1	312	4	2	2	1
1	20	12	2	1	3	1	21	2	2	3	2
1	24	10	1	1	3	1	216	4	3	1	2
1	25	22	3	1	3	4	72	3	1	3	2
2	24	17	3	2	3	2	18	2	1	1	2
2	24	17	3	2	4	2	11	1	1	1	2
1	23	9	1	2	3	1	0	1	1	1	2
2	28	18	3	2	4	1	9	1	1	1	2
2	21	11	2	1	5	1	29	3	2	2	1
2	20	8	1	1	1	1	336	4	1	1	2
1	32	13	2	1	5	4	360	4	1	3	2
1	27	9	1	1	2	1	6	1	1	1	2
1	28	12	2	1	2	4	7	3	3	3	2
1	38	13	2	2	5	4	144	4	3	3	2
1	24	12	2	2	4	1	72	3	3	2	1
2	26	15	2	2	4	1	9	1	1	1	2
2	29	13	2	1	3	3	96	4	1	1	2
1	29	7	1	1	3	4	13	2	2	1	2
1	21	16	3	1	4	4	9	1	1	3	2
1	20	15	2	2	6	4	8	1	1	3	2
1	18	11	2	1	2	4	2	1	1	3	2
1	22	14	2	2	3	1	10	1	1	1	2
2	26	22	3	3	5	3	288	4	3	3	2
2	26	22	3	3	5	3	288	4	3	3	2
2	27	11	2	1	4	1	5	1	1	1	2
2	27	11	2	1	4	1	5	1	1	1	2
1	27	11	2	1	4	1	26	2	2	2	1
1	31	11	2	1	3	2	6.5	1	1	2	1
2	21	21	3	3	4	4	48	4	2	3	2
1	23	14	2	2	4	1	0	1	1	1	2
1	21	13	2	1	2	1	6	1	1	2	1
1	21	13	2	1	2	1	6	1	1	2	1
1	32	19	3	2	4	4	48	4	3	3	2
2	24	17	3	2	4	1	8	1	1	1	2
1	27	18	3	1	4	4	10	1	1	1	2
1	27	18	3	1	4	4	10	1	1	1	2
2	22	10	2	1	3	4	72	4	3	3	2
1	24	8	1	1	1	1	10	1	1	3	2

1	28	22	3	1	6	4	18	2	3	3	2
2	20	18	3	1	4	1	7	1	1	3	2
2	24	19	3	1	4	1	3	1	1	1	2
2	22	25	3	2	4	4	3	1	3	3	2
2	22	25	3	2	4	4	3	1	3	3	2
2	30	12	2	1	2	4	7	1	1	3	2
1	20	11	2	1	3	4	7	1	1	3	2
2	23	15	2	3	5	1	5.5	1	2	1	2
2	23	15	2	3	5	1	5.5	1	2	1	2
1	40	14	2	1	6	4	7	1	3	3	2
1	40	14	2	1	6	4	7	1	3	3	2
2	23	12	2	1	5	4	6	1	1	3	2
1	28	13	2	1	5	4	6.5	1	2	3	2
1	20	12	2	1	4	2	3	1	1	1	2
1	20	12	2	1	4	2	3	1	1	1	2
1	21	18	3	1	6	1	0.5	1	1	1	2
1	20	19	2	3	4	1	8	1	2	1	2
1	20	19	2	3	4	1	8	1	2	1	2
1	24	13	2	1	3	1	9	1	2	2	1
1	29	13	2	2	4	4	19	2	2	3	2
1	21	9	1	1	2	1	18	2	1	1	2
1	29	9	1	1	3	4	672	4	1	3	2
1	24	22	3	2	4	2	7	1	1	1	2
1	24	22	3	2	4	2	7	1	1	1	2
2	21	18	2	2	4	1	16	2	2	1	2
2	25	13	2	3	5	2	2	1	1	1	2
1	22	12	2	2	6	1	96	4	1	1	2
1	29	11	2	2	3	1	48	4	3	2	1
2	37	9	1	3	5	4	9	3	3	2	1
1	18	8	1	3	3	4	4	1	1	3	2
1	19	19	3	1	4	1	72	4	3	1	2
1	22	12	2	1	4	1	48	4	2	3	2
1	27	16	2	2	4	1	36	3	3	2	2
2	28	13	2	1	4	4	144	4	1	1	2
1	25	14	2	1	3	1	0	1	1	1	2
2	22	21	3	2	5	4	9	1	1	1	2
2	22	21	3	2	5	4	9	1	1	1	2
1	22	16	3	1	2	1	6	1	2	2	1
1	23	16	3	2	5	4	312	4	3	2	1
1	20	18	3	1	3	4	8	1	2	2	2
2	27	15	2	1	5	2	19	2	2	3	2
1	28	14	2	2	2	4	552	4	3	3	2
1	18	18	2	2	6	4	7	1	2	3	2
1	20	12	2	1	2	1	27	3	3	1	2
1	22	16	3	1	3	1	9	1	1	1	2
1	23	15	2	2	4	1	168	4	3	1	2
1	35	12	2	1	4	2	3	1	3	3	2
1	35	12	2	1	4	2	3	1	3	3	2
1	27	10	1	1	4	4	10	3	3	1	2
1	27	10	1	1	4	4	10	3	3	1	2

[illegible]

ROM (hrs)	Maternal s	IAP status	PVs	No. PVs	Lab Dura	Apgar at 5	Emp antib	Resus req	Resus details
	1	2	2	2	12	8	1	1	3
	2	2		2	17	8	2	2	1
26	2	1		3	11	8	1	2	1
	2	2		2	19	8	2	2	1
	2	2		1	11	9	2	2	1
4	1	1		2	21	8	1	2	1
	2	2	4	3	12	6	1	1	4
	2	2		1	16	7	2	2	1
12	2	2		1	11	8	2	2	1
	1	1		1	19	8	1	2	1
	2	1		2	15	9	1	2	1
41	2	2		2	12	8	1	2	1
	2	2		1	13	8	1	2	1
6	2	2	2	1	4	4	1	1	4
14	2	2		2	12	7	1	1	3
	2	2		1	13	9	2	2	1
3	2	2	4	2	5	8	2	2	1
	1	1			8	7	1	2	1
14	2	2	2	1	7	7	2	2	1
21	2	2	1	1	12	8	1	2	1
4	2	1	3	1	9	9	2	2	1
	2	2			0.5	8	2	2	1
4	2	2	3	1	9	8	2	2	1
5	2	2	4	2	8	8	2	2	1
	1	1	3	1		8	1	2	1
54	2	1	4	2	12	8	1	2	1
	2	2	3	1	9	8	2	2	1
	2	2	2		4	8	2	2	1
	2	2	2	1	5	8	2	2	1
	2	2				8	2	2	1
	2	2				8	2	2	1
	2	2	2			8	2	2	1
7	2	2	3	1	11	8	2	2	1
	2	2			0	9	2	2	1
	2	2			0	8	2	2	1
6	2	2	3	1	7	8	2	2	1
6	2	2	3	1	7	8	2	2	1
20	2	1	4	1	14	8	1	2	1
20	2	1	3	1	14	8	2	2	1
	2	2	1	1		9	2	2	1
	2	2	1	1	5	8	2	2	1
24	2	1	2	1	18	7	1	2	1
24	2	1	2	1	18	7	1	2	1
	2	2				8	2	2	1
	2	2	3	1	10	8	2	2	1
	2	2	3	1	8	8	2	2	1
	2	2	3	1	8	8	2	2	1
	2	2				6	1	1	4
	2	2	5	2	13	6	1	1	4

	2	2	2	1		8	2	2	1
	2	2	3	1	16	9	2	2	1
	2	2	3	1	11	9	2	2	1
	2	2	2	1		9	2	2	1
	2	2	2	1		9	2	2	1
	2	2				6	2	2	1
	2	2	5	2	18	6	1	1	3
	2	2	3	1	17	9	2	2	1
	2	2	3	1	17	9	2	2	1
	2	2				6	2	1	3
	2	2				7	1	2	1
	2	2	2	1	8	9	2	2	1
	2	2	3	1	11	9	2	2	1
4	2	2	3	1	13	9	2	2	1
4	2	2	3	1	13	9	2	2	1
3	2	2	2	1	8	9	2	2	1
8	2	2	2	3	12	8	1	2	1
8	2	2	2	3	12	7	1	1	3
43	2	1	3	1	19	9	1	2	1
	2	2	1	1		7	1	1	3
	2	1	2	1	15	7	1	1	4
	2	2	1	1		8	2	2	1
	2	2	4	2	16	7	1	1	3
	2	2	4	2	16	7	1	1	3
	2	2	3	2	19	9	1	2	1
24	2	1	2	1	18	9	2	2	1
	2	2	3	1	15	8	2	2	1
48	2	1	4	2	22	8	1	2	1
194	2	1	2	1		8	1	2	1
	2	2				7	1	1	4
8	2	2	3	1	14	9	2	2	1
	2	2			7	8	1	1	3
48	2	1	4	2	16	9	1	2	1
	2	2	2	1	15	8	2	2	1
	2	2	2	1	8	7	1	2	1
1	2	2	1	1		8	2	2	1
1	2	2	1	1		8	2	2	1
72	2	1	4	2	9	9	1	1	3
312	2	1	3	1		8	1	2	1
26	2	1	5	2		8	1	2	1
	2	2	2	1	12	7	1	1	3
	2	1	3	1		7	2	1	3
	2	2	2	1		7	2	2	1
	2	2	4	2	16	7	1	2	1
	2	2	2	1	12	8	1	2	1
	1	2	1	1	9	7	2	2	1
	2	2	4	2	13	7	1	2	1
	2	2	4	2	13	7	1	1	3
	2	2	4	2		9	1	2	1
	2	2	4	2		7	1	2	1

[illegible]

support Forr	circ support	Mech ve	Surfactant	IV line	duration	Picc line	duration	Umblical li	Duration
2	1	2	2	1	7	2		2	
3	1	2	2	2		2		2	
3	1	2	2	1	5	2		2	
3	1	2	2	1	15	2		2	
3	1	2	2	1	1	2		2	
3	1	1	2	1	19	2		2	
4	1	1	1	1	17	1	5	1	3
3	1	2	2	2		2		2	
3	1	2	2	2		2		2	
2	1	1	1	1	23	1	8	1	5
3	1	1	2	1	17	1	5	2	
3	1	2	2	1	5	2		2	
3	1	1	2	1	14	1	8	2	
2	1	2	2	1	17	1	6	2	
3	1	1	1	2	6	2		1	3
2	1	2	2	1	16	2		2	
3	1	2	2	1	17	2		2	
2	1	2	2	1	3	2		2	
3	1	2	2	1	4	2		2	
3	1	2	2	1	3	2		2	
3	2	2	2	1	10	2		2	
3	1	2	2	1	5	2		2	
3	1	2	2	2		2		2	
2	2	2	2	1	13	1	7	2	
3	2	1	2	1	10	1	8	2	
3	1	2	2	1	3	2		2	
1	1	2	2	2		2		2	
3	1	2	2	2		2		2	
3	1	1	2	1	20	1	8	2	
3	1	2	2	2		2		2	
2	2	2	1	1	14	2		2	
2	2	1	2	2	21	2	5	2	
3	1	2	2	2		2		2	
3	1	2	2	2		2		2	
3	1	2	2	2		2		2	
3	1	2	2	2		2		2	
3	1	2	2	2		2		2	
3	1	2	2	1	5	2		2	
3	1	2	2	2		2		2	
2	2	1	1	2	5	2		2	
3	1	2	2	2		2		2	
3	2	1	2	1	24	1	8	2	
3	2	1	2	1	7	1	7	2	
3	1	2	2	2		2		2	
2	1	2	2	1	3	2		2	
3	1	2	2	2		2		2	
3	1	2	2	2		2		2	
4	2	1	1	1	7	1	3	2	
4	2	1	1	1	4	1	3	2	

3	1	2	2	2		2		2	
3	1	2	2	1	6	2		2	
3	1	2	2	2		2		2	
3	1	2	2	1	4	2		2	
3	1	2	2	2		2		2	
3	1	1	2	1	20	2		2	
2	2	1	1	2	21	1	5	2	
3	1	2	2	1	3	2		2	
3	1	2	2	1	3	2		2	
4	2	1	1	1	5	1	5	2	
4	2	1	1	1	18	2		2	
3	1	2	2	2		2		2	
3	1	1	1	1	17	2		2	
3	1	2	2	1	12	1	10	2	
2	2	1	2	1	21	1	6	2	
3	1	2	2	2		2		2	
3	1	2	2	1	5	2		2	
2	2	1	2	1	25	1	8	2	
3	1	2	2	1	7	2		2	
2	1	1	2	1	5	2		2	
4	2	1	1	1	3				
3	1	2	2	2		2		2	
2	2	1	1	1	2	2		2	
2	2	1	1	1	2	2		2	
3	1	1	1	2		2		2	
2	1	1	2	1	15	2		2	
3	1	2	2	2				2	
3	1	2	2	1	3	2		2	
3	1	2	2	1	14	2		2	
4	1	1	1	1	3	2		2	
3	1	2	2	2		2		2	
2	1	2	2	1	11	1	5	2	
3	1	2	2	1	6	2		2	
2	1	2	2	2		2		2	
2	1	2	2	1	7	2		2	
4	1	1	2	1	5	1	5	2	
4	1	1	2	2		2		2	
3	1	2	2	1	5	2		2	
3	1	2	1	1	7	2		2	
3	1	2	2	2		2		2	
2	1	2	2	1	3	2		2	
2	1	1	1	1	9	2		2	
3	1	1	1	1	7	2		2	
2	2	1	1	1	5	2		2	
2	2	1	1	1	3	2		2	
3	1	2	2	1	5	2		2	
2	1	1	2	1	5	2		1	4
2	1	2	2	1	7	2		2	
3	1	2	2	2		2		2	
3	1	2	2	2		2		2	

[illegible]

Clinical dete	Sep Screen	Blood cultur	culture organi	Sepsis +	Sep/cultur	meningitis	culture
1	2	2	7	1	3		
2				1	3		
1	2	2	7	1	3		
1	1	1	4	2	1	2	2
2				1	3		
1	1	1	3	2	1	1	2
1	2	2	7	1	3	2	
2				1	3		
2				1	3		
1	1	2	7	2	2	2	2
1	1	1	4	2	1	2	2
2				1	3		
1	2	2	7	1	3		
1	1	2	7	2	2		
2				1	3		
1	1	1	5	2	1	2	2
2				1	3		
2				1	3		
1	2	2	7	1	3		
2				1	3		
1	1	2	7	2	2	2	2
2				1	3		
2				1	3		
1	2	2	7	1	3		
2				1	3		
1	1	2	7	2	2		
1	1	1	4	2	1	2	2
2				1	3		
2				1	3		
2				1	3		
1	1	1	3	2	1	2	2
2				1	3		
1	1	2	7	2	2		
1	1	1	4	2	1	1	2
2				1	3		
2				1	3		
2				1	3		
2				1	3		
2				1	3		
1	1	2	7	2	2		
2				1	3		
1	2	2	7	1	3		
2				1	3		
1	1	1	4	2	1	2	2
1	1	1	4	2	1	2	2
2				1	3		
1	2	2	7	1	3		
2				1	3		
2				1	3		
1	1	1	2	2	1	2	2
1	1	2	7	2	2	2	2

2				1	3		
1	2	2	7	1	3		
2				1	3		
2				1	3		
2				1	3		
1	1	2	7	2	2		
1	1	1	3	2	1	2	2
2				1	3		
2				1	3		
1	1	2	7	2	2		
2	2	2	7	1	3		
2				1	3		
1	1	2	7	2	2		
1	1	2	7	2	2		
1	1	1	3	2	1	2	2
2				1	3		
2				1	3		
1	1	2	7	2	2	2	2
1	2	2	7	1	3	2	2
1	2	2	7	1	3		
1	1	2	7	2	2		
2				1	3		
1	2	2	7	1	3		
1	2	2	7	1	3		
2				1	3		
1	1	2	7	2	2		
2				1	3		
2				1	3		
1	1	1	2	2	1	2	2
1	2	2	7	1	3		
2				1	3		
1	1	2	7	2	2	2	2
2				1	3		
2				1	3		
1	1	2	7	2	2	2	2
1	2	2	7	1	3		
2				1	3		
1	2	2	7	1	3		
1	1	2	7	2	2		
2				1	3		
2				1	3		
1	1	2	7	2	2		
2				1	3		
2				1	3		
1	2	2	7	1	3		
1	2	2	7	1	3		
2				1	3		
1	1	2	7	2	2		
2				1	3		
2				1	3		
1	2	2	7	1	3		
1	2	2	7	1	3		
2				1	3		
1	1	2	7	2	2		
2				1	3		
2				1	3		

[illegible]

Discharge	Alive - D 30	D 30 status	Hosp outco	Hosp stay -	Hosp stay -	Rpt adm	Reason	DOL
1	1	1	1	12		2		
1	1	1	1	8		2		
1	1	1	1	9		2		
1	1	1	1	25		2		
1	1	1	1	11		2		
2	1	3	1	40		2		
1	1	1	1	32		2		
1	1	1	1	10		2		
1	1	1	1	9		2		
2	1	4				2		
1	1	1	1	22		2		
1	1	1	1	17		2		
1	1	1	1	23		2		
2	1	4				2		
1	1	1	1	19		2		
2	1	4	1	35		2		
2	1	4	1	35		2		
1			1	12				
2	2	2	2		7	2		
1	1	1	1	10		1	1	17
1	1	1	1	12		2		
1	1	1	1	8		2		
1	1	1	1	7		2		
2	1	4	1	32		2		
2	2	2	2		10	2		
1	1	1	1	11		2		
1	1	1	1	7		2		
1	1	1	1	9		1	1	14
2	1	4	1	35		2		
1	1	1	1	21		2		
1	1	1	1	22		2		
2	1	3	1	44		2		
1	1	1	1	9		2		
1	1	1	1	9		2		
1	1	1	1	9		2		
1	1	1	1	9		2		
1	1	1	1	9		2		
1	1	1	1	9		2		
1	1	1	1	21		2		
1	1	1	1	19		2		
2	1	4				2		
2	2	2	2		7	2		
1	1	1	1	8		2		
1	1	1	1	14		2		
1	1	1	1	14		2		
1	1	1	1	14		2		
2	2	2	2		7	2		
2	2	2	2		4	2		

1	1	1	1	10		2		
1	1	1	1	10		2		
1	1	1	1	10		2		
1	1	1	1	13		2		
1	1	1	1	13		2		
1	1	1	1	21		2		
2	2	2	2		22	2		
1	1	1	1	17		2		
1	1	1	1	17		2		
2	2	2	2		5	2		
2	1	4	1	37		2		
1	1	1	1	22		2		
2	2	2	2		21	2		
2	1	4				2		
2	2	2	2		21	2		
1	1	1	1	15		2		
1	1	1	1	27		2		
2	1	3	1	27		1	1	29
2	1	3	1	14		1	1	27
1	1	1	1	27		2		
2	2	2	2		4	2		
1	1	1	1	21		2		
2	2	2	2		1	2		
2	2	2	2		2	2		
1	1	1	1	10		2		
1	1	1	1	10		2		
1	1	1	1	24		2		
1	1	1	1	9		2		
1	1	1	1	17		2		
2	2	2	2		3	2		
1	1	1	1	8		2		
2	1	4	1	35		2		
2	1	4	1	37		2		
1	1	1	1	11		2		
1	1	1	1	17		2		
1	1	1	1	12		2		
1	1	1	1	12		2		
1	1	1	1	26		2		
1	1	1	1	9		2		
1	1	1	1	10		2		
1	1	1	1	12		2		
1	1	1	1	18		2		
1	1	1	1	25		2		
1			1	12				
2	2	2	2		3	2		
2	1	3	1	17		1	1	27
1	1	1	1	26		2		
1	1	1	1	26		2		
1	1	1	1	21		2		
1	1	1	1	21		2		

[illegible]

Abs days	Antibiotics	Sepsis +	Sep epi in Hc	W 1TI		W1T2		W1T3	
5	1	1		36.8	1	36.2	1	36.5	1
	2	1		36.5	1	36	1	36.4	1
5	1	1		36.8	1	36.3	1	36.5	1
14	1	2	1	36.8	1	36	1	36.2	1
	2	1		36.5	1	35.8	2	36.4	1
27	1	2	3	36.5	1	36.1	1	36	1
10	1	1	1	36.9	1	34.2	3	36.3	1
	2	1		36.8	1	36.2	1	36.5	1
	2	1		36.5	1	36.3	2	36.5	1
18	1	2	2	36.5	1	35.1	3	36.5	1
17	1	2	1	36.5	1	35.1	3	36.4	1
5	1	1		36.5	1	35.8	2	36.5	1
3	1	1		36.5	1	35.1	3	36.3	1
17	1	2	2	36.5	1	35.1	3	36.5	1
	2	1		36.5	1	35	3	36.5	1
14	1	2	1	36.5	1	35.1	3	36.5	1
17	1	1	1	36.5	1	35.1	3	36.5	1
3	1	1		36.5	1	36.5	1	36.5	1
4	1	1	1	36.4	1	35.8	2	35.8	2
3	1	1	1	36.5	1	36.4	1	36.5	1
10	1	2	1	36.5	1	36.1	1	36.5	1
5	1	1		36.5	1	36.4	1	36.5	1
	2	1		36.5	1	36.1	1	36.5	1
10	1	2	1	36.5	1	35.4	3	36.4	1
10	1	2	1	36.8	1	32.1	3	36.5	1
3	1	1		36.5	1	36.5	1	36.5	1
	2	1		36.5	1	36.5	1	36.5	1
	2	1		36.5	1	36.4	1	36.5	1
18	1	2	1	36.5	1	34.9	3	36.5	1
	2	1		36.5	1	35.4	3	36.5	1
14	1	2	1	36.5	1	36	1	36.5	1
21	1	2	3	36.5	1	35.1	3	36.5	1
	2	1		36.5	1	36.4	1	36.5	1
	2	1		36.5	1	36.2	1	36.5	1
	2	1		36.5	1	36.2	1	36.5	1
	2	1		36.5	1	36.5	1	36.5	1
	2	1		36.5	1	36.5	1	36.5	1
7	1	2	1	36.5	1	36.5	1	36.5	1
	2	1		36.5	1	36.2	1	36.3	1
3	1	1		36.5	1	36	1	36.5	1
	2	1		36.5	1	36.5	1	36.5	1
24	1	2	3	36.5	1	34.1	3	36.5	1
7	1	2	1	36.5	1	35.4	3	36.5	1
	2	1		36.5	1	36.2	1	36.5	1
3	1	1		36.5	1	36.5	1	36.5	1
	2	1		36.5	1	36.5	1	36.5	1
	2	1		36.5	1	36.5	1	36.5	1
7	1	2	1	36.5	1	36	1	36.5	1
4	1	2	1	36.5	1	36	1	36.5	1

	2	1		36.5	1	36.5	1	36.5	1
3	1	1		36.5	1	36.5	1	36.5	1
	2	1		36.5	1	36.5	1	36.5	1
	2	1		36.5	1	36	1	36.5	1
	2	1		36.5	1	36	1	36.5	1
20	1	2	2	36.5	1	36.4	1	36.5	1
21	1	2	2	36.5	1	35.8	2	36.5	1
	2	1		36.5	1	36.5	1	36.5	1
	2	1		36.5	1	36.5	1	36.5	1
5	1	2	1	36.5	1	35.8	2	36.5	1
14	1	1	2	36.5	1	35.8	2	36.5	1
10	1	1	1	36.5	1	36.4	1	36.5	1
16	1	2	2	36.5	1	35.4	1	36.5	1
10	1	2	1	36.5	1	35.2	3	36.5	1
21	1	2	2	36.5	1	34.1	3	36.5	1
	2	1		36.1	1	36.1	1	36.5	1
5	1	1		36.5	1	35.1	3	36.5	1
25	1	2	2	36.5	1	35.1	3	36.5	1
18	1	1	2	36.5	1	36.2	1	36.5	1
3	1	1		36.5	1	35.2	3	36.5	1
3	1	2	1	36.5	1	35.2	3	36.5	1
	2	1		36.5	1	36.4	1	36.5	1
2	1	1		36.5	1	35.2	3	36.5	1
2	1	1		36.5	1	35.2	3	36.5	1
	2	1		36.5	1	36.5	1	36.5	1
7	1	2	1	36.5	1	36.2	1	36.5	1
10	1	1	1	36.5	1	36.4	1	36.5	1
3	1	1		36.5	1	36.5	1	36.5	1
14	1	2	1	36.5	1	36.5	1	36.5	1
3	1	1		36.5	1	35.1	3	36.5	1
	2	1		36.5	1	36.4	1	36.5	1
10	1	2	1	36.5	1	36.5	1	36.5	1
5	1	1		36.4	1	35.1	3	36.5	1
3	1	1		36.5	1	36.1	1	36.5	1
7	1	2	1	36.5	1	36.4	1	36.5	1
3	1	1		36.5	1	35.4	3	36.5	1
	2	1		36.5	1	35.1	3	36.5	1
5	1	1		36.5	1	35.8	2	36.5	1
7	1	2	1	36.5	1	36.3	1	36.5	1
3	1	1		36.5	1	35.7	1	36.5	1
3	1	1		36.5	1	36.2	1	36.5	1
7	1	2	1	36.5	1	35.8	2	36.5	1
3	1	1	2	36.5	1	36.4	1	36.5	1
5	1	1		36.1	1	36.5	1	36.5	1
3	1	1		36.5	1	36.2	1	36.5	1
3	1	1	1	36.4	1	36.4	1	36.5	1
5	1	1		36.5	1	35.1	3	36.5	1
7	1	2	1	36.5	1	35.8	2	36.5	1
	2	1		36.5	1	35.8	2	36.5	1
7	1	1	1	36.5	1	35.8	2	36.5	1

[illegible]

W 2T1		W2T2		W2T3		W3T1		W3T2	
36.5	1	36.3	1	36.5	1	36.4	1	36.5	1
36.5	1	36.1	1	36.5	1	36.5	1	36.1	1
36.4	1	36.2	1	36.5	1	36.5	1	36.1	2
36.5	1	36.3	1	36.5	1	36.4	1	36	2
36.4	1	35.9	2	36	1	36.5	1	36.2	2
36.8	1	36.3	1	36.5	1	36.1	1	35.2	3
36.5	1	35.1	3	36.5	1	36.5	1	35.5	3
35.4	1	35.2	3	35.2	3	35.9	1	35.4	3
36.5	1	36.1	1	36.4	1	36.5	1	36.4	1
36.5	1	34.2	3	36.5	1	36.5	1	35.8	2
36.5	1	36.4	1	36.5	1	36.5	1	35.3	3
36.5	1	35.4	3	36.4	1	36.5	1	35.2	3
36.5	1	35.6	2	36.5	1	36.5	1	35.8	2
36.4	1	36.5	1	35.4	3	36.5	1	35.6	2
36.5	1	34.9	3	36.5	1	36.5	1	34.7	3
36.5	1	35.2	3	36.5	1	36.5	1	36	1
36.5	1	35	3	36.5	1	36.5	1	35.8	2
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	36.2	1	36.5	1	36.8	1	36.4	1
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	34.2	3	36.5	1	36.5	1	35.1	3
36.5	1	36.3	1	36.5	1	36.5	1	36.5	1
36.5		36.4	1	36.5	1	36.5	1	36.3	1
36.5	1	35.2	3	36.5	1	36.5	1	35.1	3
36.5	1	34	3	36.4	1	36.4	1	35	3
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	35.7	2	35.4	2	36.5	1	35.7	2
36.5	1	35.4	2	36.5	1	36.5	1	36.3	1
36.5	1	35.3	3	36.5	1	36.5	1	36.1	1
36.4	1	36.2	1	36.5	1	36.5	1	36	1
36.5	1	35.8	3	36.2	1	36.5	1	36.2	1
36.5	1	36.2	1	36.5	1	36.4	1	36.5	1
34.5	3	35	3	35	3	35	3	35	3
34.5	3	35	3	35	3	35	3	35	3
36.2	1	36.2	1	36.2	1	35.9	2	35.2	2
36.2	1	36.2	1	36.2	1	35.9	2	35.2	2
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	35.8	2	36	1	36.5	1	36.5	1
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	35.6	2	36.5	1	36.5	1	35.3	3
36	1	35.2	3	35.9	2	36.2	1	35.2	3
36.5	1	35.5	2	36.5	1	36.5	1	35.2	3
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	36.5	1	36.5	1	35.4	3	35.8	2
36.5	1	35.1	3	36.5	1	35.8	2	35.4	3
36.5	1	36.2	1	36.5	1	36.5	1	36.3	1
36.5	1	36.5	1	36.1	1				

35.6	2	35	3	35	3	35.1	3	35	3
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	36.5	1	36.5	1	35.8	2	35.4	3
36.5	1	36.1	1	36.5	1	36.5	1	36.3	1
36.5	1	36.1	1	36.5	1	36.5	1	36.3	1
36.5	1	36.3	1	36.5	1	36.5	1	36.5	1
36.5	1	35.2	3	35.6	2	36.5	1	35.9	2
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	36.1	1	36.5	1	36.5	1	36	1
36.5	1	36.1	1	36.5	1	36.5	1	36	1
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	35.9	2	36.5	1	36.5	1	36.2	1
36.5	1	35.4	3	36.5	1	36.5	1	36.2	1
36.5	1	35.4	3	36.5	1	36.5	1	36	1
36.5	1	36.2	1	36.5	1	36.5	1	35.4	1
36.5	1	35.4	3	36.5	1	36.5	1	36.2	1
36.5	1	35.1	3	36.5	1	36.5	1	36	1
36.5	1	36	1	36.5	1	36.5	1	36.1	1
36.5	1	35.4	3	36.5	1	36.5	1	36.1	1
36.5	1	35.1	3	36.5	1				
36.5	1	36.2	1	36.5	1	36.5	1	36.4	1
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	36.4	1	36.5	1	36	1	36.3	1
36.5	1	36.2	1	36.5	1	36.5	1	36.1	1
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	35.3	3	36.5	1				
36.5	1	36.2	1	36.5	1	36.5	1	36.5	1
36.5	1	35.9	2	36.3	1	36.5	1	36.4	1
36.5	1	35.6	2	36.5	1	36.5	1	35.6	2
36.5	1	36	1	36.5	1	36.1	1	35.2	3
36.5	1	36.2	1	36.5	1	36.5	1	36.4	1
36.5	1	36.2	1	36.5	1	36.5	1	36.4	1
36.2	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	36.2	1	36.5	1	36.3	1	36.5	1
36.5	1	36.4	1	36.5	1	36.5	1	36.4	1
36.5	1	36.2	1	36.5	1	36.5	1	36	1
36.5	1	36.1	1	36.5		36.5	1	36.4	1
36.5	1	35.4	3	36.4	1	36.5	1	36.2	1
36.2	1	36	1	36.5	1	36.4	1	36.1	1
36.5	1	36.3	1	36.5	1	36.5	1	36.2	1
36.5	1	35.2	3	36.5	1				
36.5	1	36.5	1	36.5	1	36.5	1	36.5	1
36.5	1	35.4	3	36.5	1	36.6	1	35.8	2
35.8	2	36.5	1	36.5	1	36.6	1	36.2	1
36.5	1	36.2	1	36.5	1	36.5	1	36.5	1
36.5	1	36.2	1	36.5	1	36.5	1	36.5	1

[illegible]

W3T3		AX- cc-B	Axil - B	AX- cc-24	Axil-24	AX- cc-48	Axil - 48
36.5	1	0	7	1000	1	0	7
36.3	1	100000	1	100000	1	0	7
36.5	1	100000	1	100	1	0	7
36.5	1	100000	1	100000	2	100	2
36.5	1	100000	1	0	7	0	7
36.4	1	10000	1	10000	2	0	7
36.5	1	0	7	0	7	0	7
36.2	1	0	7	100	2	0	7
36.5	1	100	1	0	7	100000	1
36.5	1	1000	2	1000	1	100000	1
36.5	1	1000	2	100000	2	100000	1
36.5	1	1000	1	10000	2	100	1
36.5	1	0	7	100	2	1000	1
36.5	1	10000	1	100	1	100	1
36.5	1	1000	1	1000	2	100	1
36.5	1	10000	1	1000	1	1000	2
36.3	1	100	1	10000	1	10000	1
36.5	1	100	1	1000	1	0	7
36.5	1	0	7	0	7	100	1
36.5	1	100	1	0	7	100	2
36.5	1	1000	2	100000	2	10000	2
36.5	1	0	7	100	1	100000	1
36.5	1	0	7	0	7	0	7
36.5	1	0	7	100000	1	10000	1
36.5	1	100	2	10000	2	100000	2
36.5	1	100	1	0	7	1000	1
36.5	1	100	1	100	1	100	2
35.4	2	100	1	10000	1	100000	2
36.2	1	0	7	0	7	100	1
36.4	1	0	7			1000	2
36.5	1	0	7			100000	1
36.5	1	0	7	100000	1		
36.5	1	0	7	100	2		
35	3	0	7	0	7	100000	2
35	3	10000	2	100000	2	0	7
35.9	2	100	1	100000	1	100000	2
35.9	2	100	1	100000	2	100000	1
36.5	1	100000	2	100000	2	0	7
36.5	1	100	1	0	7	100000	2
36.5	1	100	2	10000	2	100	2
36.5	1	10000	1	100	1	0	7
36.5	1	0	7	0	7	100000	2
36.3	1	0	7	0	7	100000	2
35.4	3	100	1	10000	1	100000	1
36.5	1	0	7	100	1	0	7
36	1	0	7	100000	1	100	1
36.5	1	0	7	100000	1	100000	1
36.5	1	1000	1	0	7	1000	1
		100	1	100	2	10000	2

35	3	0	7	100	2	0	7
36.5	1	0	7	100	1	0	7
35.4	1	0	7	1000	1	1000	2
36.5	1	0	7	0	7		
36.5	1	100	2	100000	1		
36.3	1	0	7	0	7	100	2
36.5	1	1000	2	0	7	10000	1
36.5	1	10000	1	0	7	100	2
36.5	1	100	1	0	7	0	7
36.5	1	0	7	100	2	10000	1
36.5	1	100	1	0	7	0	7
36.5	1	100	1	100	1	10000	2
36.5	1	10000	1	1000	2	100	2
36.5	1	100	2	0	7	0	7
36.5	1	10000	2	1000	2	100	2
36.2	1	100	1	100000	2	1000	2
36.5	1	0	7	0	7	0	7
36.5	1	0	7	0	7	100	2
36.5	1	10000	1	1000	2	10000	2
36.5	1	10000	1	100000	2	0	7
		100	2	100000	2	10000	2
36.5	1	1000	2	100	2	0	7
		10000	1				
		1000	2	1000	2		
36.5	1	10000	2	10000	2	0	7
36.5	1	0	7	10000	2	1000	2
36.5	1	0	7	0	7	100	2
36.5	1	0	7	10000	2	10000	2
36.5	1	0	7	10000	2	10000	2
		0	7	0	7	0	7
36.5	1	100000	2				
36.5	1	0	7	0	7	0	7
36.5	1	100000	1	1000	2	100	2
36.5	1	100000	1	100000	2	100000	2
36.5	1	100000	1	100000	2	100000	2
36.5	1	0	7	1000	1	1000	2
36.5	1	0	7	10000	2	100	1
36.5	1	0	7	100000	2	100000	2
36.5	1	0	7	100	2	10000	1
36.5	1	100	2	100	2	0	7
36.5	1	0	7	0	7	0	7
36.5	1	0	7	0	7	10000	2
36.3	1	100000	2			1000	2
36.5	1	10000	2	10000	2	1000	2
		10000	2	10000	2	100000	2
36.5	1	100000	2	10000	2	1000	2
36.5	1	0	7	10000	2	0	7
36.5	1	0	7	0	7	1000	2
36.5	1	100	1	0	7	0	7
36.5	1	1000	1	1000	1	10000	1

[illegible]

AX- cc- D7	Axil - D7	GR-cc- B	Groin - B	GR-cc- 24	Groin - 24	GR-cc- 48	Groin- 48
0	7	0	7	0	7	100	1
0	7	100000	1	10000	2	0	7
100000	2	100000	2	1000	2	100000	1
100	2	100000	1	10000	1	0	7
1000	2	100000	1	100	2	0	7
100	2	100000	2	100000	2	100	2
100000	2	0	7	0	7	0	7
100	2	0	7	1000	1	100	1
1000	1	0	7	0	7	10000	2
100000	1	1000	1	1000	1	10000	1
100000	1	100	2	100000	1	100000	2
100000	1	100	1	10000	1	100000	1
10000	1	0	7	100	2	100000	2
1000	1	1000	1	100	1	0	7
0	7	1000	1	0	7	100000	2
100	1	1000	1	1000	1	10000	1
1000	1	100	1	10000	1	1000	1
0	7	100	1	1000	1	0	7
		0	7	0	7	100	1
100000	2	0	7	0	7	100	2
1000	2	100	2	100000	2	10000	2
100000	1	0	7	100	1	100000	1
100000	1	0	7	0	7	0	7
10000	1	0	7	100000	1	10000	1
100000	2	100	2	1000	2	100000	1
0	7	100	1	0	7	1000	2
100000	1	100	1	100	1	100	1
100000	2	0	7	10000	1	100000	2
10000	1	0	7	0	7	100	1
100000	2	0	7			10000	1
100000	1	0	7			100000	1
100000	1	0	7	1000	1		
0	7	0	7	1000	1		
0	7	0	7	0	7	100000	2
1000	1	10000	2	0	7	100000	2
0	7	0	7	100000	2	100000	2
0	7	0	7	100000	2	100000	2
10000	2	100000	1	100000	2	0	7
0	7	0	7	0	7	100	2
100000	2	10000	2	1000	2	1000	2
0	7	100	1	100000	2	1000	2
0	7	0	7	0	7	10000	2
100000	2	0	7	0	7	100000	2
100000	1	10000	1	10000	1	100000	1
100000	1	0	7	0	7	0	7
100000	1	0	7	100000	2	100000	1
100000	1	0	7	10000	2	100000	1
0	7	10000	1	0	7	0	7
		0	7	100	2	10000	2

100000	2	0	7	100	2	0	7
0	7	100	1	100	1	100	2
0	7	100	1	0	7	10000	2
0	7	100000	1	1000	2		
1000	2	0	7	0	7		
1000	2	0	7	0	7	100	2
10000	1	0	7	1000	1	100000	7
0	7	100	1	0	7	100	2
0	7	1000	1	0	7	0	7
		0	7	100000	2	10000	1
0	7	0	7	0	7	0	7
0	7	100	1	100	1	10000	2
1000	1	10000	1	1000	2	10000	2
0	7	100000	1	0	7	0	7
100	1	100	1	1000	2	100000	2
100000	2	10000	1	100000	2	10000	1
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1000	2	0	7	1000	2	1000	2
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0	7	10000	1	10000	2	0	7
		100	2	100000	2	100000	2
0	7	10000	2	10000	2	0	7
		0	7				
		0	7	100	1		
0	7	1000	2	100000	2	0	7
100	1	0	7	100000	2	100000	2
100000	1	0	7	0	7	10000	2
1000	1	0	7	10000	2	10000	2
1000	1	0	7	0	7	0	7
		0	7	0	7	0	7
100	2	10000	2				
0	7	0	7	0	7	100000	2
100	2	1000	2	10000	2	100	2
100000	2	100000	2	10000	2	100000	2
100000	2	100000	2	10000	2	100000	2
1000	2	0	7	10000	2	10000	
100	1	0	7	100000	2	1000	2
100000	1	0	7	100	2	100000	2
100000	1	0	7	100	2	10000	1
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0	7	0	7	0	7	0	7
0	7	0	7	0	7	100000	1
100	2	0	7			100	1
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1000	2	0	7	100000	2	100000	2
0	7	10000	1	100	2	0	7
1000	1	10000	1	100	1	100	1
0	7	1000	1	0	7	0	7
100	1	100000	1	100	1	100	1

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